21st Century Oncology

TRC TRANSLATIONAL RESEARCH CONSORTIUM

State of the art connection between immunotherapy and radiation therapy: Where we are now and where we are going?

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tgen

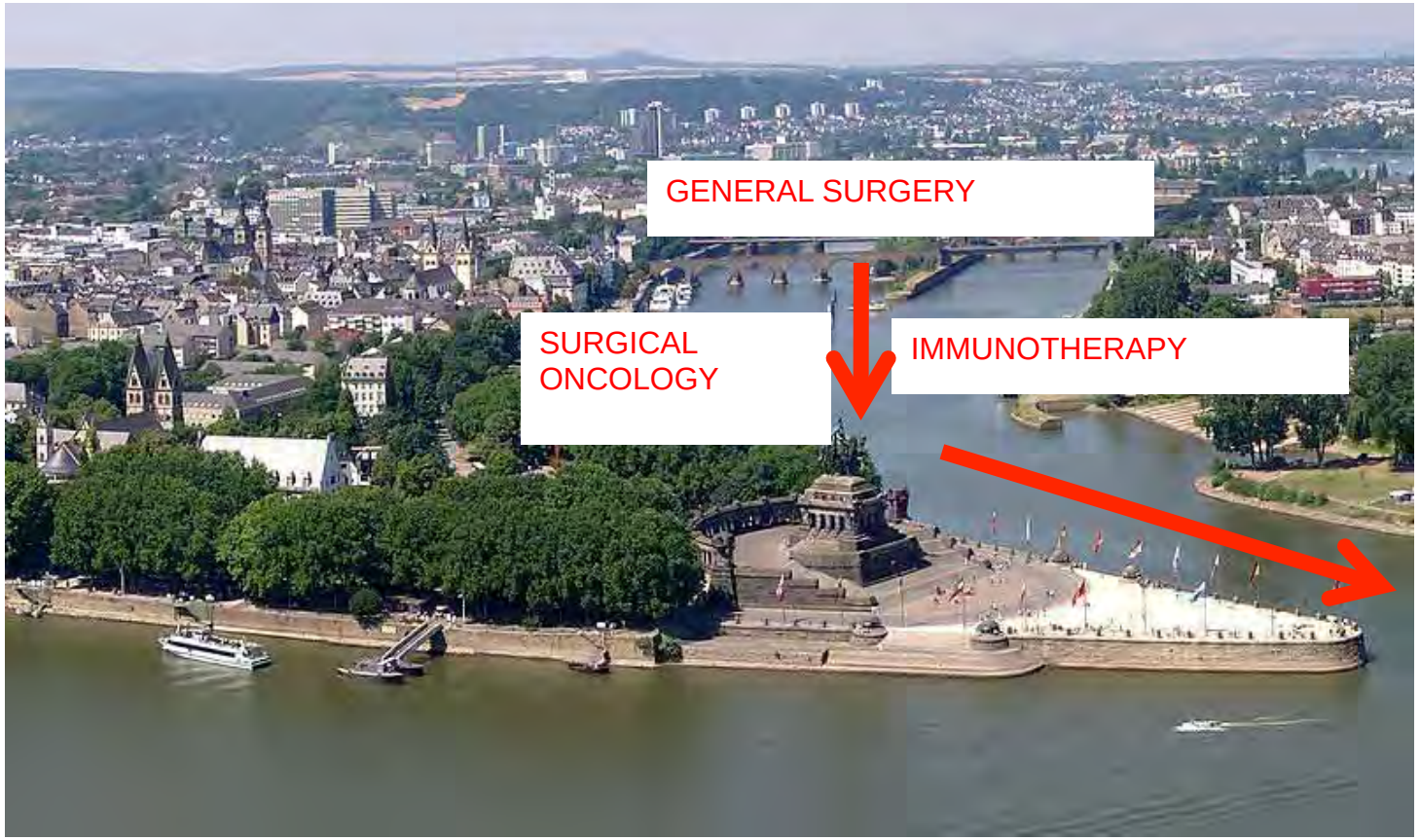
TD2 TRANSLATIONAL DRUG DEVELOPMENT

21st Century Oncology

21st Century TRC Mission:

- Building novel approaches combining radiation therapy and other therapies to elicit *radiation induced personalized systemic therapy*

The Journey to Date



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21st Century Oncology

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Bedside to bench and back again: how animal models are guiding the development of new immunotherapies for cancer

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Abstract: Immunotherapy using adoptive cell transfer is a promising approach that can result in the regression of bulky, invasive cancer in some patients. However, currently available therapies remain less successful than desired. To study the mechanisms of action and possible improvements in cell-transfer therapies, we use a murine model system with analogous components to the treatment of patients. T cell receptor transgenic CD8$^+$ T cells (pmel-1) specifically recognizing the melanocyte differentiation antigen gp100 are adoptively transferred into lympho-depleted mice bearing large, established, 14-day subcutaneous B16 melanoma (0.5–1 cm in diameter) on the day of treatment. Adoptive cell transfer in combination with interleukin interleukin-2 or interleukin-15 cytokine administration and vaccination using an altered form of the target antigen, gp100, can result in the complete and durable regression of large tumor burdens. Complete responders frequently develop autoimmunity with vitiligo at the former tumor site that often spreads to involve the whole coat. These findings have important implications for the design of immunotherapy trials in humans. *J. Leukoc. Biol.* 76: 333–337; 2004.

Key Words: *IFN-γ · MHC · interleukin · melanoma · adoptive cell transfer · vaccination · active immunization · cytokine · tumor*

THE PROBLEM

Metastatic melanoma is a significant public health concern in the United States with increasing incidence and mortality rates over the past several decades. The estimated lifetime risk of melanoma in the United States is approximately one in 55 males and one in 82 females [1]. Approximately 55,100 cases of invasive melanoma are estimated for 2004 [1]. It is estimated that 7910 patients with metastatic melanoma will die of their disease this year [1].

The ability to successfully and consistently treat advanced melanoma has been an elusive goal. At initial presentation to physicians, the majority of patients will have skin disease only without palpable nodes or evidence of distant metastases [2]. Most patients will undergo surgical treatment by wide local excision alone; additionally, sentinel lymph node biopsy and/or regional nodal dissection may be used. After surgical resection to render patients clinically free of disease, clinical observation, adjuvant therapy using interferon-α (IFN-α) or experimental therapies may be recommended [3]. Despite these interventions, some patients will progress to develop metastatic disease and succumb to their illness [4]. Thus, new therapies capable of treating advanced metastatic melanoma are urgently needed.

IMMUNOTHERAPY TO DESTROY BULKY, INVASIVE CANCER

A wide variety of therapies for metastatic melanoma have been attempted including surgery, radiotherapy, chemotherapy, and biological therapy. In some instances, immunotherapy can be used effectively to treat patients with metastatic disease. Complete and durable regression of stage IV melanoma has been reported using interleukin-2 (IL-2)-based immunotherapy alone [5]. At our institution, 182 patients with metastatic melanoma were treated with high-dose intravenous (i.v.) bolus IL-2 between September 1985 and November 1996. As of June 2003, 12 patients (7%) were complete responders, and 16 patients (9%) were partial responders for a total response rate of 15%. All patients who were complete responders beyond 18 months (83%) remained free of disease as of June 2003.

Although a limited number of patients can be cured of metastatic melanoma solely using high-dose IL-2, the response rate still remains low. This has led to the use of IL-2 in conjunction with other treatment modalities, including vaccines, monoclonal antibodies, and the adoptive transfer of T lymphocytes. The generation of highly active, tumor-specific lymphocytes and their administration in large numbers to patients are the basis of adoptive cell-transfer therapy [6].

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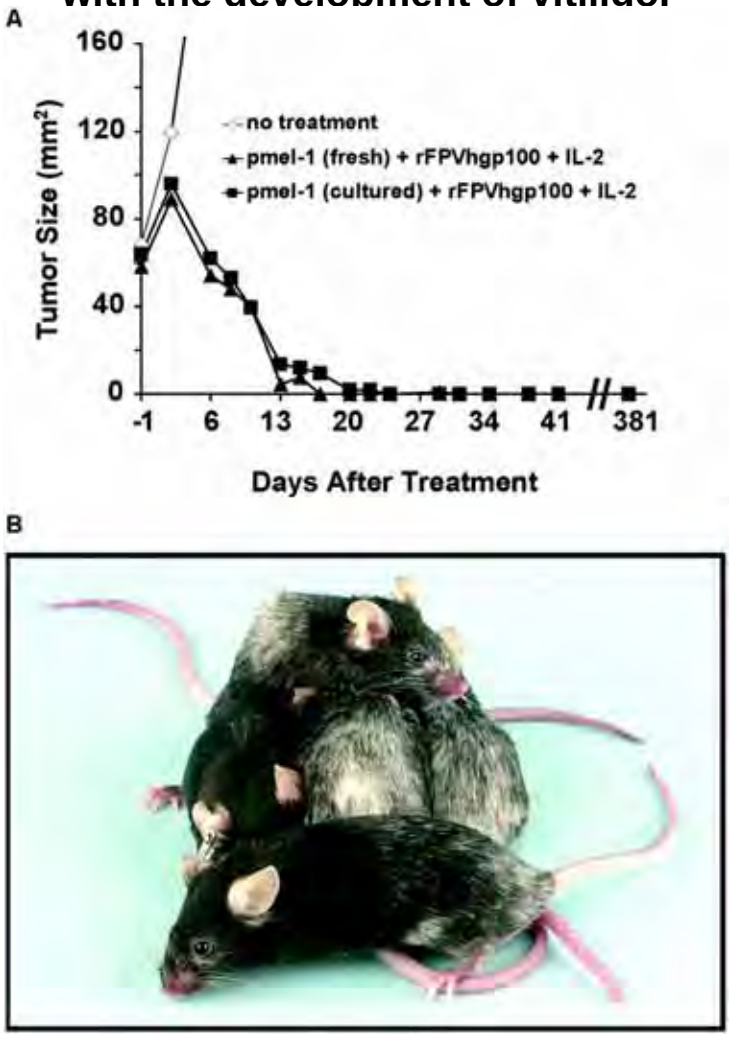
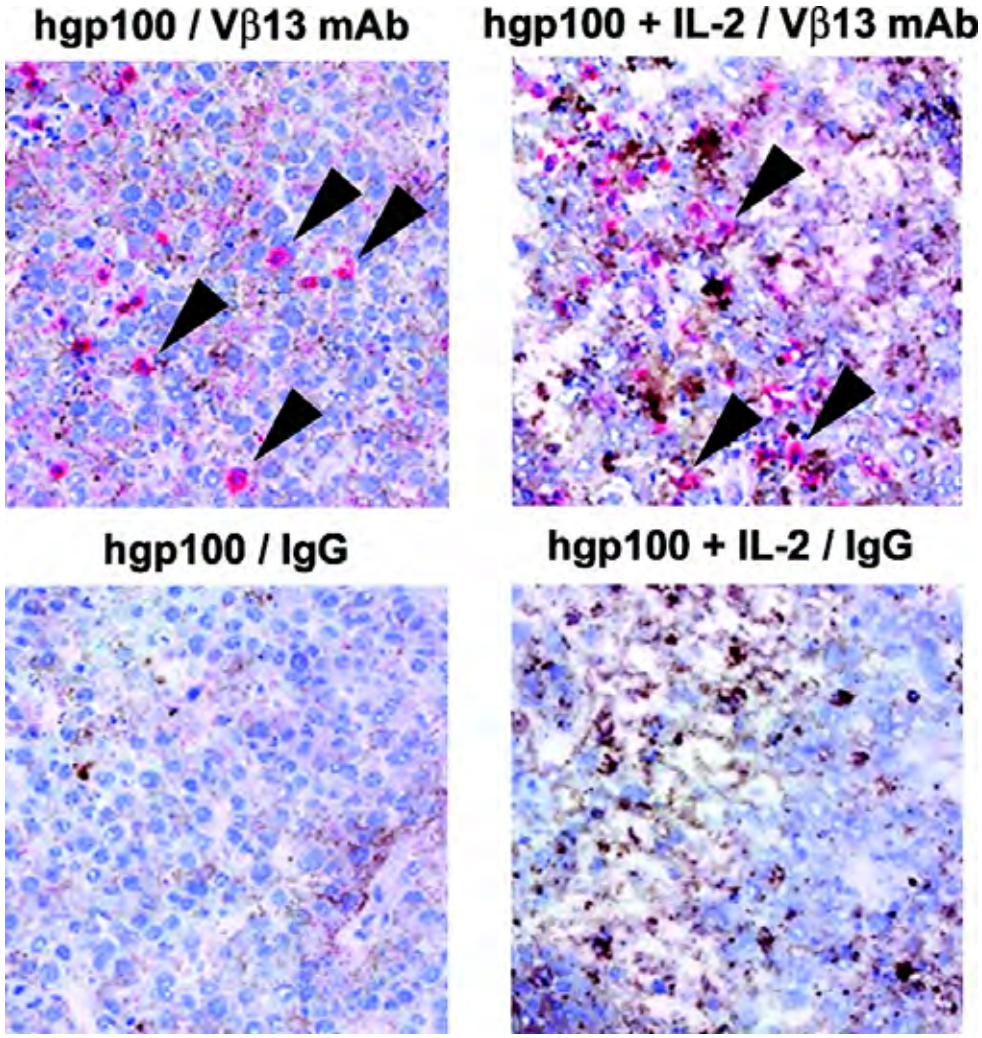
•Proc Natl Acad Sci U S A. 2008 Jun 10;105(23):8061-6. Epub 2008 Jun

Long-term (>1 yr) survival of mice bearing large, established B16 tumors after treatment with adoptive transfer of tumor-specific T cells combined with vaccination and IL-2 is associated with the development of vitiligo.



JEM 2003;198:569-580

Histological analysis reveals presence of Vβ13+ T cells in tumors and demonstrates the activating effects of IL-2.



JEM 2003;198:569-580

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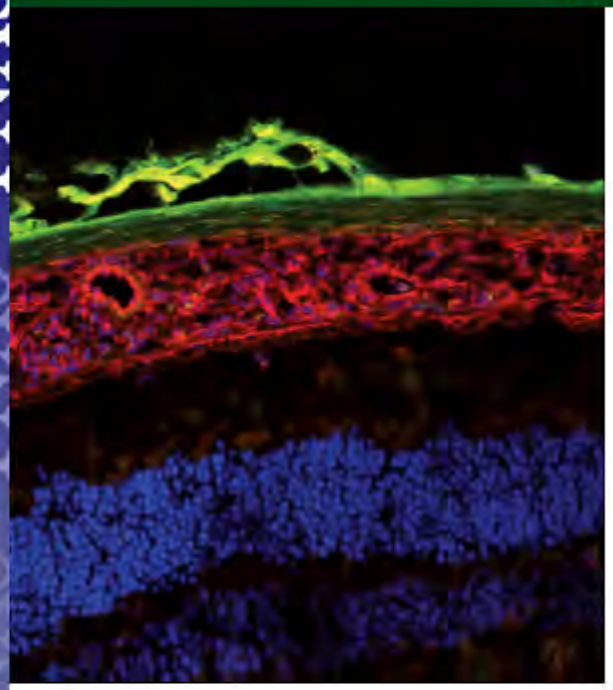
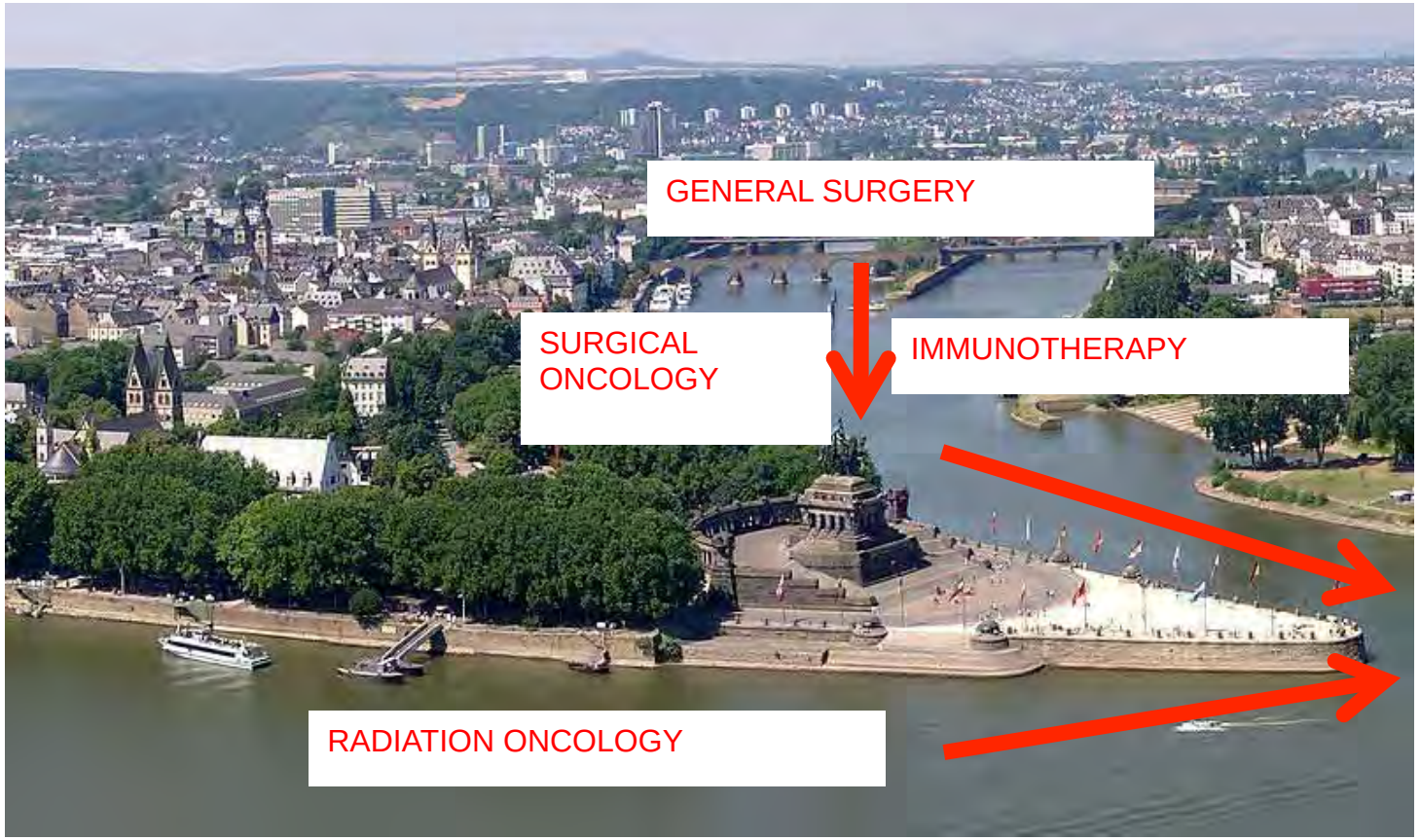


Cover image: The eye is thought to be privileged from immune attack. However, after potent cancer immunotherapy targeting melanocytes, the eye is extensively damaged. This severe autoimmunity is associated with up-regulation of MHC class I molecules (red) in the choroid layer. In their article on pages 8061–8066, Palmer *et al.* explore the relationship between anti-tumor treatment and autoimmunity. Image courtesy of Douglas C. Palmer.

From the Cover

The Journey to Date



Direct Interaction between Radiation and Tumor Cells

- Classical teaching suggests conventional fractionation external beam radiation / low dose rate brachytherapy cause dsDNA breaks causing mitotic death

Direct Interaction between Radiation, Tumor Cells, and the Immune System

- Aspects of direct immune modulation of immune cells by RT
  - Increased expression of MHC class I and coaccessory molecules following radiation of both tumor and host cells
  - RT can trigger signals that stimulate toll-like receptor 4 on antigen presenting dendritic cells (DCs)
  - Irradiation of DCs can enhance presentation of antigenic peptides
  - Direct phenotypic change of tumor cells, rendering them more susceptible to vaccine-mediated T-cell killing
  - Radiation-induced changes to tumor immune microenvironment can promote greater infiltration of immune effector cells

Mechanisms of Radiation Driven Immunotherapy (RDI)

- RT can cause “danger signals”
  - – Cause the release of tumor antigens and molecules in Damage-Associated Molecular Patterns (DAMPs), which can:
    - Induce the expression of cytokines and chemokines, and release inflammatory mediators
  - – Create an inflammatory setting via:
    - DC maturation, induction of apoptosis, necrosis, cell surface molecules, and secretory molecules

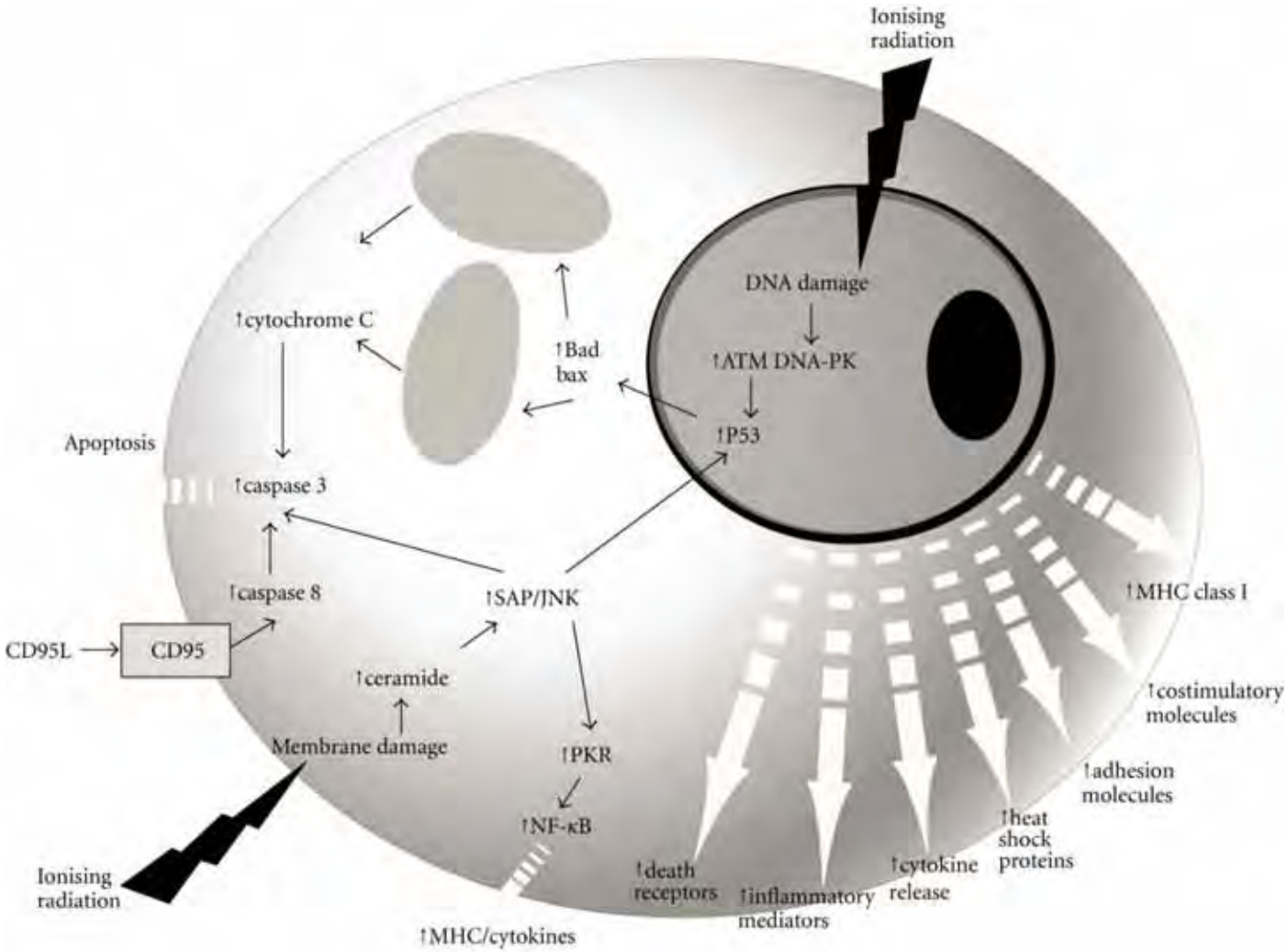


Figure 1: Apoptosis can be initiated by SABR-induced DNA damage and upregulation of the p53 tumor suppressor gene. In addition, apoptosis can be triggered by SABR-induced damage to the cellular lipid membrane, which can induce ceramide formation and activate the SAPK/JNK signaling pathway. Thus, SAPK/JNK can upregulate PKR expression, which can induce MHC and cytokines via NF-κB. SABR can induce cellular expression of MHC Class I, adhesion molecules, costimulatory molecules, heat shock proteins, inflammatory mediators, immunomodulatory cytokines, and death receptors.

Finkelstein, S.E., Timmerman, R., McBride, W.H., Schaue, D., Hoffe, S.E., Mantz C.A., Wilson, G.D. The Confluence of Stereotactic Ablative Radiotherapy (SABR) and Tumor immunology. Clinical and Developmental Immunology 2011;2011:439752

Cancer Control
Journal of the Moffitt Cancer Center

From Bench to Bedside: Bringing Immunotherapy Into the Clinic

Immunotherapy is an exciting approach that can result in the regression of bulky, invasive cancer in some patients. Much progress has been made in our understanding of the role of the host immune response in affecting tumor progression and response to various treatments. Through these advances, novel immunotherapies have been introduced into the clinic.

In this issue of *Cancer Control*, experts review the latest clinical and therapeutic aspects of emerging immunotherapies for numerous disease sites.

We have seen a recent explosion of agents for metastatic castrate-resistant prostate cancer. In the lead article, Dr Shore and colleagues review data relating to the potential pharmacodynamic biomarkers associated with the immunotherapy sipuleucel-T, as well as considerations for patient selection and for sequencing this agent with other prostate cancer treatments. Indeed, sipuleucel-T is the first autologous cellular immunotherapy approved by the US Food and Drug Administration for the treatment of asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer. In this platform, peripheral blood mononuclear cells (antigen-presenting cells and T cells) are obtained from each patient via leukapheresis and treated ex vivo with PA2024, a fusion protein consisting of prostatic acid phosphatase/granulocyte-macrophage colony-stimulating factor antigen. In three phase III trials, sipuleucel-T showed improvement in overall survival. This sets the stage for further approval of novel immune-modulating approaches. There are numerous possible directions for future development, including treatment of less advanced prostate cancer populations, combination treatment, and immune modulation.

In the second article in this issue, Dr Soliman provides an overview of the available data of breast cancer regarding the immune-modulating effects of both current and novel treatments. With respect to breast cancer, there is increasing evidence to support the theory that some breast tumors may be more immunogenic than others; tumors that elicit more potent cytotoxic T-cell responses appear to have a more favorable prognosis and respond better to chemotherapy than do less immunogenic tumors. This is coupled with a realization that standard treatments rely in part on their immunogenic effects for their success in eliminating lesions. New immunomodulatory agents and vaccines that can reverse underlying immunosuppression caused by established tumors are currently in development. Combining these novel agents for breast cancer with current therapies may boost their efficacy.

Lung cancer presents a difficult problem as the most common cause of cancer-related deaths in the United States. It is without perfect solutions as traditional chemotherapy fails to provide long-term benefit for many patients. New innovative approaches are desperately needed to improve overall survival beyond the current standard of care. Dr Hall and colleagues review the most recent clinical trials using immunotherapy techniques to treat both non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). For NSCLC, phase II clinical trials have examined allogeneic vaccines that target various epitopes including but not limited to mucin 1, epidermal growth factor, and melanoma-associated antigen 3. Vaccine approaches against these antigens are undergoing phase III trials. In addition, autologous cellular therapy approaches directed against transforming growth factor beta-2 and a recombinant protein with antitumor properties have also shown promise in prolonging survival in NSCLC in phase II trials. The monoclonal antibodies ipilimumab, BMS-936558 (anti-PD-1), and BMS-936559 (anti-PD-L1) have appeared to lead to enhanced T-cell–mediated antitumor effects with objective responses in early-phase clinical trials. Studies for SCLC have been more limited.

Esophageal, gastroesophageal, gastric, liver, pancreatic, and colorectal gastrointestinal malignancies have been targeted for immune treatment as these sites represent the highest incidence among human cancers worldwide. The majority of gastrointestinal cancers are frequently unresectable at the time of diagnosis; there have only been modest improvements in survival in this setting with the addition of traditional modalities such as chemotherapy and radiation therapy. Dr Toomey and associates review current immunotherapeutic strategies to improve outcomes. To date, monoclonal antibody therapy is the only immunotherapy approved by the US Food and Drug Administration for gastrointestinal cancers. Initial trials validating novel immunotherapeutic approaches for gastrointestinal malignancies, including vaccination-based and adoptive cell therapy strategies, have adequately demonstrated safety and the induction of antitumor immune responses.

The next article focuses on brain cancer. Despite improvements in surgical technique, radiation therapy delivery, and options for systemic cytotoxic therapy, the median survival for newly diagnosed glioblastoma multiforme patients remains poor at 15 months with trimodality therapy. As Dr Marsh and colleagues review, antitumor vaccines (dendritic and formalin-fixed) have demonstrated clinical efficacy in phase I and II trials with mild toxicity, suggesting that innate immune responses can be amplified and directed against these tumors. An alternative approach using suicide gene therapy (gene-mediated cytotoxic therapy) employing viral vectors has also shown efficacy in completed phase I and ongoing phase II trials; neural stem cells are also being investigated as vectors. Thus, the phase I and II data suggest that immunologic therapies can produce meaningful and sometimes durable responses in the treatment of glioblastoma multiforme with mild toxicity compared to other standard therapies.

In the current treatment paradigms for leukemias, hematopoietic stem cell transplant (HSCT) is considered the best option with a curative potential. Dr Brayer and associates summarize the recent advances in the field of immunotherapy for leukemia. With respect to passive immunotherapy, recent improvements in chimeric T-cell antigen receptor technology have been employed. In active immunotherapy, various clinical studies of peptide vaccination strategies focusing on molecular targets such as the Wilms' tumor gene 1 (WT1), proteinase 3 (PR3), and receptor for hyaluronan acid-mediated motility (RHAMM) suggest the immune system has the capacity to recognize and react to leukemic cells mounting inflammatory and CD4 T-cell responses to complement and support cytotoxic activity.

Finally, we discuss radioimmunotherapy. This approach has been approved for the treatment of B-cell non-Hodgkin lymphomas in the United States for over a decade. Development of radioimmunotherapy agents for advanced-stage solid malignancies has engendered renewed interest. Dr Tomblyn and coauthors examine available evidence for the preclinical and clinical development of these agents for a variety of solid tumors, including colorectal, breast, prostate, ovarian, pancreatic, hepatocellular, and primary brain tumors. Novel radioimmunotherapy agents are in active clinical investigation, either as single agents or combined with radiosensitizing chemotherapy or with external beam radiotherapy. Antibody (and antibody fragment) design and availability have improved, with fewer side effects than more traditional cytotoxic systemic therapy. Radionuclides such as alpha-emitters offer increased antitumor potency with reduced toxicity.

In summary, immunotherapeutic options for cancer are rapidly expanding. Our improved understanding of immune biology has resulted in an explosion of novel agents over the last few years. The exciting display of ongoing clinical trials and investigational drugs in immunotherapy, many of which have a novel mechanism of action, may shift the landscape of current cancer care. These new immunotherapies, used alone or in combination with other standard modalities such as radiation[1,2] or chemotherapy,[3] may lead to less toxic regimens for more patients. Optimal immunotherapeutic management requires a personalized approach tailored to the unique clinical status of each patient. Coordination of care using a multidisciplinary approach involves immunotherapists, medical oncologists, radiation oncologists, surgeons, and radiologists to achieve maximal therapeutic benefits. In this way, we will continue building immunotherapeutic bridges from the bench to bedside.

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Table 1: Combination Trials. Trials Combining Radiotherapy and Immunotherapy in Patients.

Study	Treatment	Eligibility	Outcomes	Toxicities
[11] Finkelstein et al. n=5	Intratumoral dendritic cell injection in addition to hormonal therapy and radiation	Non-metastatic prostate cancer with 2 year recurrence risk ≥30% ; HLA-A2⁺ subjects only	Safety and tolerability, with some demonstration of response to antigen	Mild pain scores/ difficulties in assessing the appropriate dose and introduction time of DCs
[24] Finkelstein et al. n=17	Intratumoral dendritic cell injection in conjunction with fractionated external beam radiation (EBRT) as neo-adjuvant treatments	High-grade soft tissue Sarcoma (STS) >5 cm	Well tolerated with 9 patients developing tumor specific immune responses. Overall, treatment led to accumulation of T cells in the tumor	Post-operative complications in 5/17 patients as well as some toxicity observed during the merged treatment
[25] Gulley et al. n=30	Poxviral vaccines encoding prostate-specific antigen (PSA) in conjunction with radiotherapy, or radiotherapy only	Non-metastatic prostate cancer	Majority of patients receiving the combination treatment generated a PSA-specific cellular immune response to the vaccine	Dose reductions due to grade 3 toxicities and other factors. Many subjects had injection site reactions
[26] Okawa et al. n=61	Vaccine utilizing a biological response modifier prepared from Lactobacillus Casei YTT9018, used in combination with radiation or radiation alone	Carcinoma of the uterine cervix	The vaccine served as an effective adjuvant immunotherapeutic agent with the combination treatment showing additional tumor reduction and enhanced therapeutic effects	Fever was observed in 6 patients of the 30 eligible cases. Reports of redness, induration, and swelling were reported as well
[27] Chi et al. n=14	Intratumoral injections of autologous immature DCs in concomitance with radiotherapy	Advanced/metastatic stage hepatoma not suitable for surgery or transarterial embolization	Demonstration that the combination treatment is safe and can induce tumor-specific and innate immunity	Of the 12 patients who completed the study, several had issues including grade 1-2 fever with chills, grade 1 fatigue, or mild myalgia/arthralgia
[28] Seung et al. n=12	Varying doses of stereotactic body radiation therapy (SBRT) followed by high-dose interleukin-2 (IL-2)	Metastatic melanoma or renal cell carcinoma patients who had received no previous medical therapy for metastatic disease	Safe administration of the combination treatment with immune monitoring displaying greater frequency of proliferating CD4 (+) T cells	Anticipated toxicities from IL-2 were observed including hypotension, fever, arthralgia, and many others. Mental status changes were noted
[29] Kim et al. n=15	TLR9 agonist injection paired with local radiation	Mycosis fungoides (MF) subjects who failed at least one standard therapy	Well tolerated treatment with mild adverse effects with a trend toward greater reduction of CD25 (+) and Foxp3 (+) T cells	All patients elicited injection site reactions with one patient being dismissed from the study. All patients reported flu-like symptoms
[30] Lechleider et al. n=18	Vaccine utilizing recombinant vaccinia virus in sequence with radiation therapy	Non-metastatic prostate cancer/ same criteria as Gulley et al. (above)	Safe administration with the combination treatment inducing prostate-specific immune responses	The majority of patients had ≤ grade 2 injection-site reactions, with other patients reporting a variety of issues
[31] Finkelstein et al. n=29	Hypofractionated Radiotherapy with concurrent interferon-alfa-2b (IFN)	Stage III Nodal disease involving the axilla, neck, or groin characterized as advanced based on defined criteria	Combination treatment provided reasonable in-field control in patients at high risk of regional failure.	3 patients withdrew due to IFN toxicity and another 2 patients experienced grade 3 skin toxicity

Agassi, A.M., Myslicki F.A, Shulman J.M.et al
The Promise of combining radiation therapy and immunotherapy: morbidity and toxicity. 2014 Future Medicine

Local radiotherapy plus intratumoral syngeneic dendritic cell injection can mediate apoptosis/cell death and immunological tumor eradication in murine models. A novel method of coordinated intraprostatic, autologous dendritic cell injection together with radiation therapy was prospectively evaluated in five HLA-A2$^+$ subjects with high-risk, localized prostate cancer, using androgen suppression, 45 Gy external beam radiation therapy in 25 fractions over 5 weeks, dendritic cell injections after fractions 5, 15 and 25 and then interstitial radioactive seed placement. Serial prostate biopsies before and during treatment showed increased apoptotic cells and parenchymal distribution of CD8$^+$ cells. CD8$^+$ T-cell responses to test peptides were assessed using an enzyme-linked immunosorbent spot IFN-γ production assay, demonstrating some prostate cancer-specific protein-derived peptides associated with increased titer. In conclusion, the technique was feasible and well-tolerated and specific immune responses were observable. Future trials could further test the utility of this approach and improve on temporal coordination of intratumoral dendritic cell introduction with particular timelines of therapy-induced apoptosis.

KEYWORDS: immunotherapy · prostate · radiation therapy

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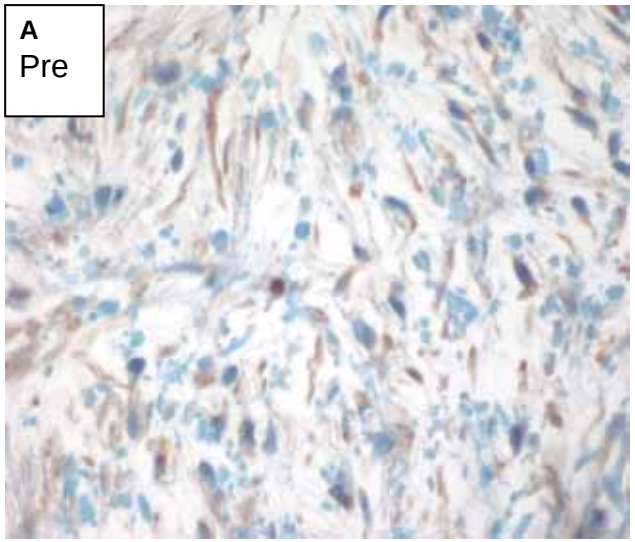
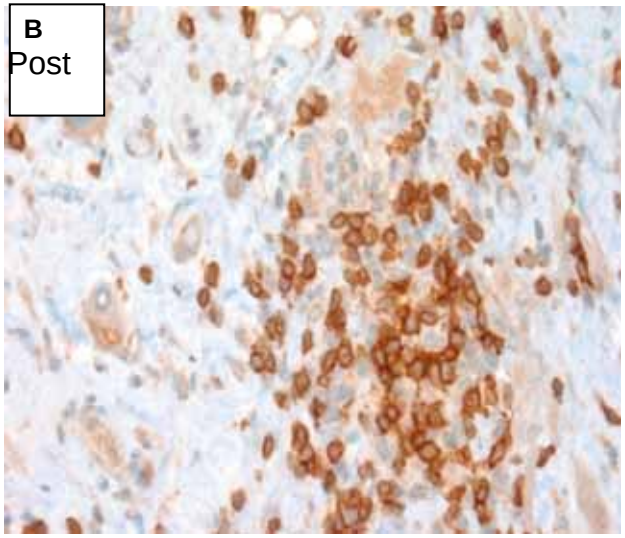
21st Century Oncology

Future Medicine fsg

Histologic Assessment





- Representative biopsy specimens prior to treatment (A), and post-treatment (B) stained with an anti-CD3 mAb
  - Prior to treatment very few, or no T cells were seen within STS
  - Post treatment at resection, extensive T cell infiltration was seen

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0360-3016/$ - see front matter

ELSEVIER

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CLINICAL INVESTIGATION

COMBINATION OF EXTERNAL BEAM RADIOTHERAPY (EBRT) WITH INTRATUMORAL INJECTION OF DENDRITIC CELLS AS NEO-ADJUVANT TREATMENT OF HIGH-RISK SOFT TISSUE SARCOMA PATIENTS

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Purpose: The goal of this study was to determine the effect of combination of intratumoral administration of dendritic cells (DC) and fractionated external beam radiation (EBRT) on tumor-specific immune responses in patients with soft-tissue sarcoma (STS).

Methods and Material: Seventeen patients with large (>5 cm) high-grade STS were enrolled in the study. They were treated in the neoadjuvant setting with 5,040 cGy of EBRT, split into 28 fractions and delivered 5 days per week, combined with intratumoral injection of 10^7 DCs followed by complete resection. DCs were injected on the second, third, and fourth Friday of the treatment cycle. Clinical evaluation and immunological assessments were performed.

Results: The treatment was well tolerated. No patient had tumor-specific immune responses before combined EBRT/DC therapy; 9 patients (52.9%) developed tumor-specific immune responses, which lasted from 11 to 42 weeks. Twelve of 17 patients (70.6%) were progression free after 1 year. Treatment caused a dramatic accumulation of T cells in the tumor. The presence of $CD4^+$ T cells in the tumor positively correlated with tumor-specific immune responses that developed following combined therapy. Accumulation of myeloid-derived suppressor cells but not regulatory T cells negatively correlated with the development of tumor-specific immune responses. Experiments with ^{111}In labeled DCs demonstrated that these antigen presenting cells need at least 48 h to start migrating from tumor site.

Conclusions: Combination of intratumoral DC administration with EBRT was safe and resulted in induction of antitumor immune responses. This suggests that this therapy is promising and needs further testing in clinical trials design to assess clinical efficacy. © 2011 Elsevier Inc.

Sarcoma, Dendritic cells, Tumor immunity, Combined treatment.

INTRODUCTION

Intratumoral administration of dendritic cells (DC) is one of the promising methods of induction of therapeutic antitumor immune responses. The main advantage of this approach is that a large variety of tumor-associated antigens present in tumors can be used. In addition, patients do not need to be selected or excluded based on human leukocyte antigen (HLA) type or the expression of specific antigens. However, immunotherapy alone rarely causes curative antitumor effects as the manipulation of tumor microenvironment is necessary to potentiate the effect of DC administration (1). Ionizing radiation presents one such powerful intervention. Radiation can not only kill tumor cells releasing tumor antigens, but can also exert various immunomodulatory effects including induction of the expression of cytokines, chemokines, and release of inflammatory mediators (2–4). It also increases the permeability of the local vasculature that leads to recruitment of circulating leukocytes into surrounding tissues including antigen-presenting cells and effector T cells (5–7).

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This work was presented at the 51st ASTRO meeting and was awarded clinical winner of the resident clinical/basic science research award.

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S.E.F., C.I., and M.M.B. contributed equally to this work.

Conflict of interest: none

Acknowledgments—This manuscript is dedicated in memory of Erin Bryant, our nurse, colleague, and friend.

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From Bench to the Bedside … and Back Again

Clinical opportunities in combining immunotherapy with radiation therapy

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Preclinical work in murine models suggests that local radiotherapy plus intratumoral syngeneic dendritic cells (DC) injection can mediate immunologic tumor eradication. Radiotherapy affects the immune response to cancer, besides the direct impact on the tumor cells, and other ways to coordinate immune modulation with radiotherapy have been explored. We review here the potential for immune-mediated anticancer activity of radiation on tumors. This can be mediated by differential antigen acquisition and presentation by DC, through changes of lymphocytes' activation, and changes of tumor susceptibility to immune clearance. Recent work has implemented the combination of external beam radiation therapy (EBRT) with intratumoral injection of DC. This included a pilot study of coordinated intraprostatic, autologous DC injection together with radiation therapy with five HLA-A2(+) subjects with high-risk, localized prostate cancer; the protocol used androgen suppression, EBRT (25 fractions, 45 Gy), DC injections after fractions 5, 15, and 25, and then interstitial radioactive implant. Another was a phase II trial using neo-adjuvant apoptosis-inducing EBRT plus intra-tumoral DC in soft tissue sarcoma, to test if this would increase immune activity toward soft tissue sarcoma associated antigens. In the future, radiation therapy approaches designed to optimize immune stimulation at the level of DC, lymphocytes, tumor and stroma effects could be evaluated specifically in clinical trials.

Keywords: dendritic cells, immunotherapy, radiation effects, stereotactic radiosurgery, immune modulation

INTRODUCTION

RADIATION EFFECTS

A conventional view of radiation is an immune attenuator. In this perspective, damage, and destruction represent the conventional view of radiation effects on living tissues – whether they are tumor, normal stroma, and parenchyma, or leukocytes. In the medical application of therapeutic radiation, this is a measured induction of apoptosis and other cell death within a carefully defined volume. The impact of radiation on leukocytes can be viewed in similarly detrimental terms, whether attenuating lymphocyte numbers as tolerable side effect (Ishihara et al., 2008; Lissoni et al., 2003) a therapeutic effect, such as part of an allogeneic transplant protocol (Wei et al., 2004; Gupta et al., 2011), or precipitating a secondary malignancy (Bell et al., 1962). The measurement of accumulated radiation injuries, such as micronuclei and DNA breakage in circulating lymphocytes, has been proposed as a direct assay of individuals' relative radiosensitivity (Minucci et al., 2009; Lang et al., 2010; Ishihara et al., 2012); that sensitivity can be relevant to either toxicity or to treatment efficacy.

We focus here on the effect of radiation on the bilateral relationship of tumor with the immune system, not just on the effects of radiation on the tumor or on the leukocytes, separately. Considered in isolation, radiation to any particular cell could be anticipated to have a detrimental impact. However, there is an opportunity in the interplay of tumor cell death, induced antigen expression on tumor cells, and inflammatory signals from the irradiated volume which affect lymphocyte and dendritic cell (DC) activation. **Figure 1** contrasts the perspectives of isolated versus system effect of irradiation. Immunotherapeutic impacts can be coordinated with therapeutic tumor irradiation. In this way, the whole therapeutic effect can exceed the sum of its parts.

PROCESSES OF CELLULAR IMMUNITY

Physiologic process of antigen presentation and lymphocyte activation are complex processes, and subject to modulation because of the tumor microenvironment (Fricke and Gabrilovich, 2006). Immature myeloid cells acquire antigen, whether by vaccination or through phagocytosis of material in the tumor microenvironment. These cells then mature, with acquisition of cell surface proteins such as MHC class I and II on which peptides derived from the antigen source can be presented, to interact with particular antigen-specific idiotypic receptors on T lymphocytes (discussed, for example, by Liao et al., 1994). Other maturational markers such as CD80, CD86 facilitate costimulation interactions, particularly the process of activation versus tolerogenic influence on those lymphocytes (these illustrated in Topalian et al., 2012, where the focus is on the PD-1/PDL-1 interaction, for example). The interaction of lymphocytes with the antigen-presenting cells, occurs in lymph nodes to which the DC migrate as part of the maturation process, and the subsequent potential anticancer effect of lymphocytes then is a consequence of lymphocytes' expansion within the lymph node, circulation and penetration into the tumor mass. Other lymphocyte pathways, such as natural killer (NK) cells, may be influenced by T cell activation and the

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21C TRC Animal Radiation-Imaging-Immunotherapy Experimental Laboratory (*ARIEL*) @ TD2

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TD2 TRANSLATIONAL DRUG DEVELOPMENT

21st Century Oncology

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TRC TRANSLATIONAL RESEARCH CONSORTIUM

ARIEL

- Cancer pharmaceutical development company, Translational Drug Development (TD2), and the Translational Research Consortium (TRC), the research arm of the largest radiation oncology provider, 21st Century Oncology, have teamed up to enable rapid testing of anti-cancer drugs combined with radiation therapy

tgen

TD2 TRANSLATIONAL DRUG DEVELOPMENT

21st Century Oncology Footprint

TRC TRANSLATIONAL RESEARCH CONSORTIUM

21st Century Oncology

TRC TRANSLATIONAL RESEARCH CONSORTIUM

ARIEL

- The TRC has installed a new, state-of-the-art small animal radiation research (SARRP) platform with TD2 that enables investigators to mimic the radiation therapy process used when treating patients with cancer

tgen

TD2 TRANSLATIONAL DRUG DEVELOPMENT

21st Century Oncology

TRC TRANSLATIONAL RESEARCH CONSORTIUM

ARIEL

- The Animal Radiation-Imaging-Immunotherapy Experimental Laboratory (***ARIEL***) houses the SARRP research platform, which provides the most advanced experimental platform for testing the efficacy of new radiation techniques and potential new drugs that, together, could enhance the standard of treatments for cancer

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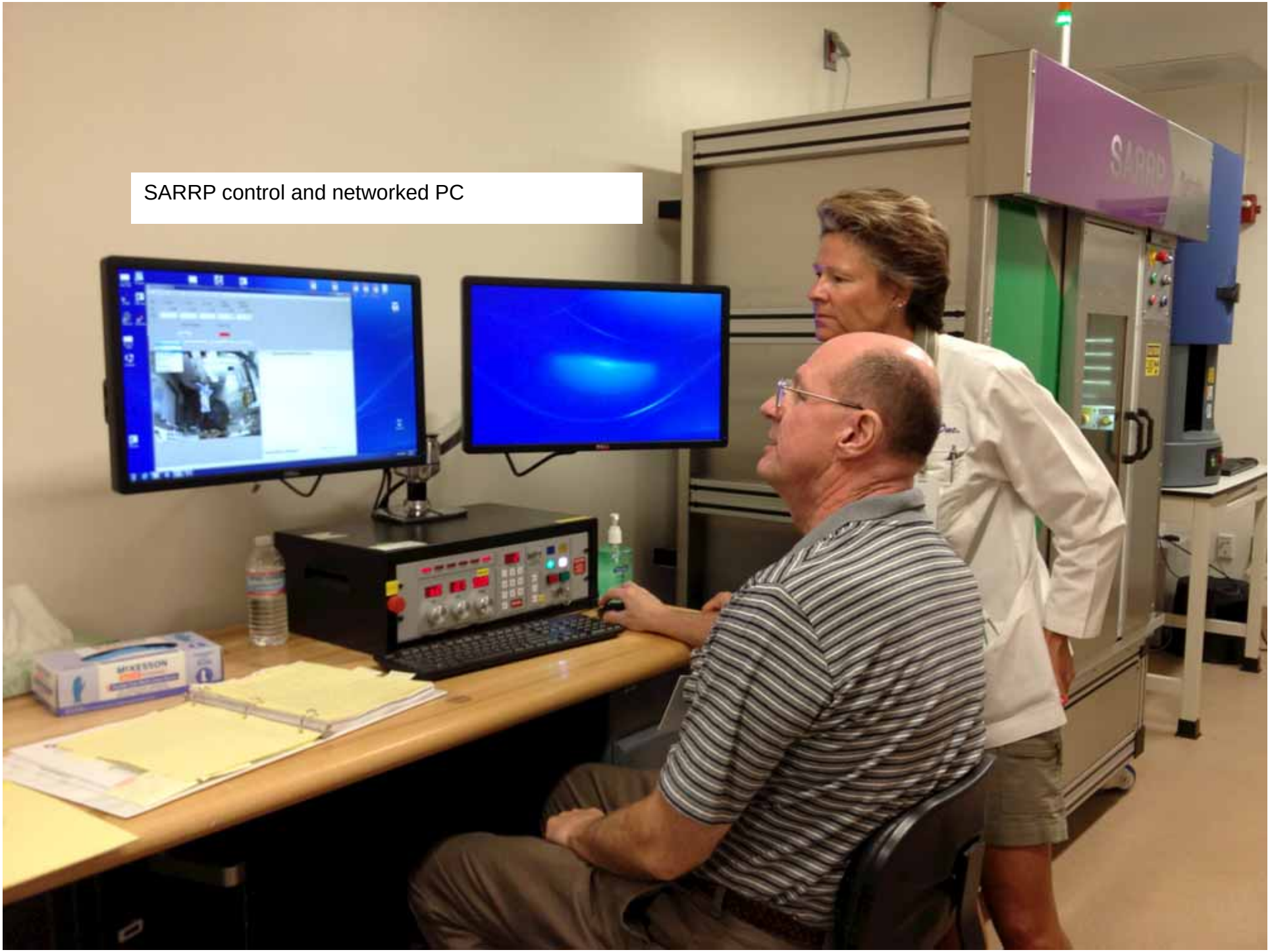
TD2 TRANSLATIONAL DRUG DEVELOPMENT

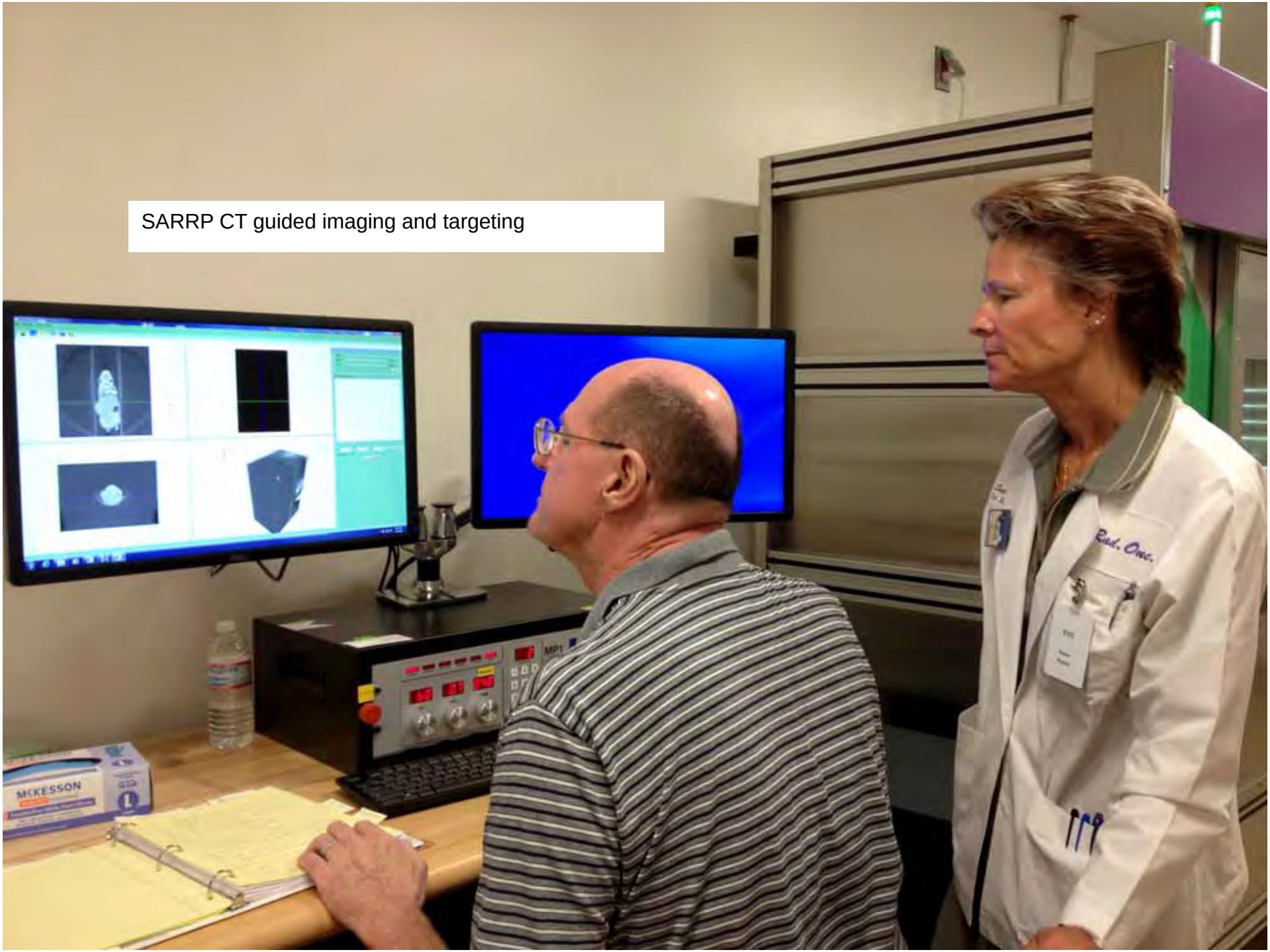
SARRP small animal radiation platform
in purpose-designed Pb cabinet

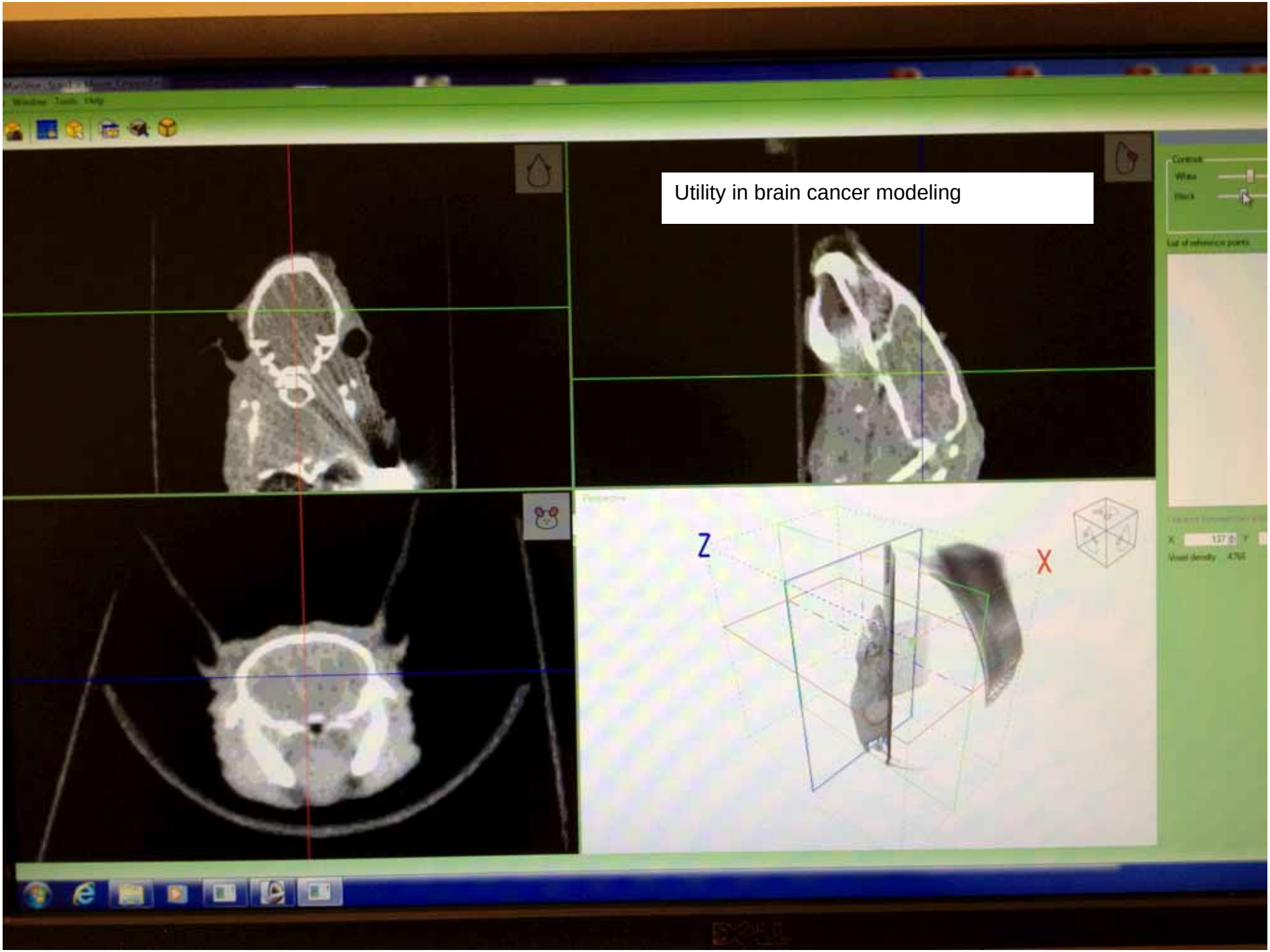
Designated room in TD2 vivarium

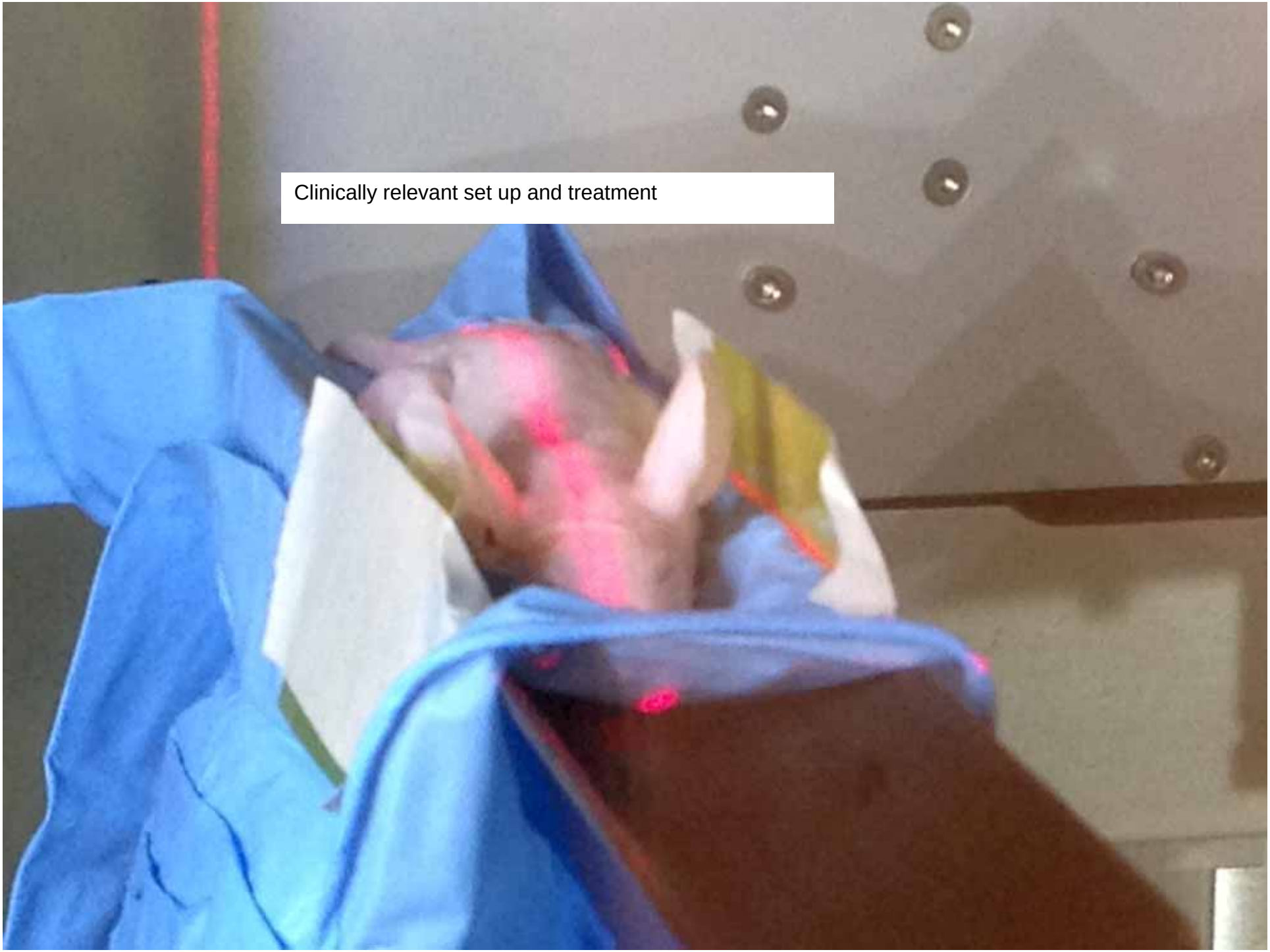


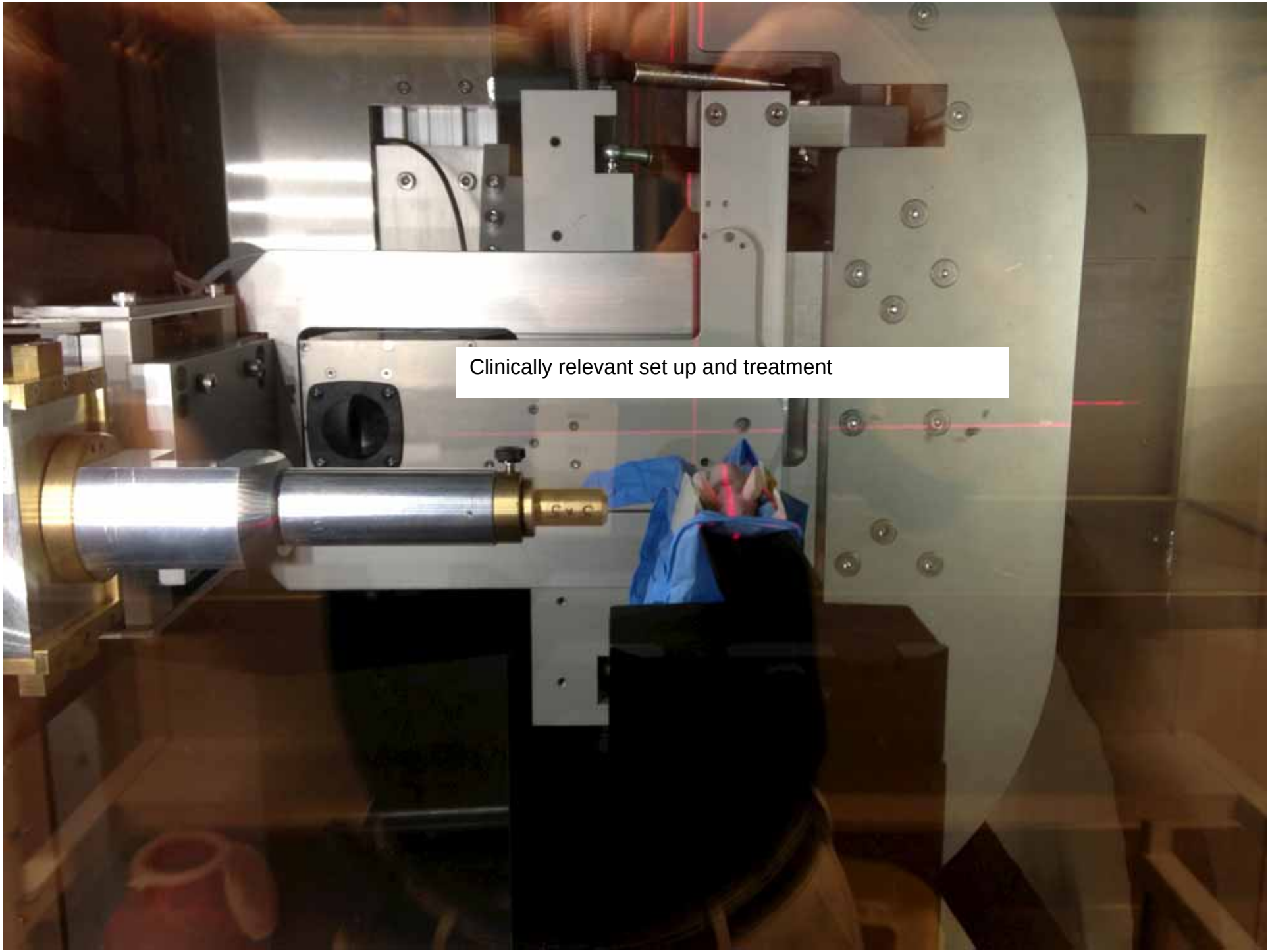
SARRP control and networked PC

Video monitoring of experimental technique


SARRP CT guided imaging and targeting
MCKESSON


Utility in brain cancer modeling
Z
X


Clinically relevant set up and treatment


Clinically relevant set up and treatment

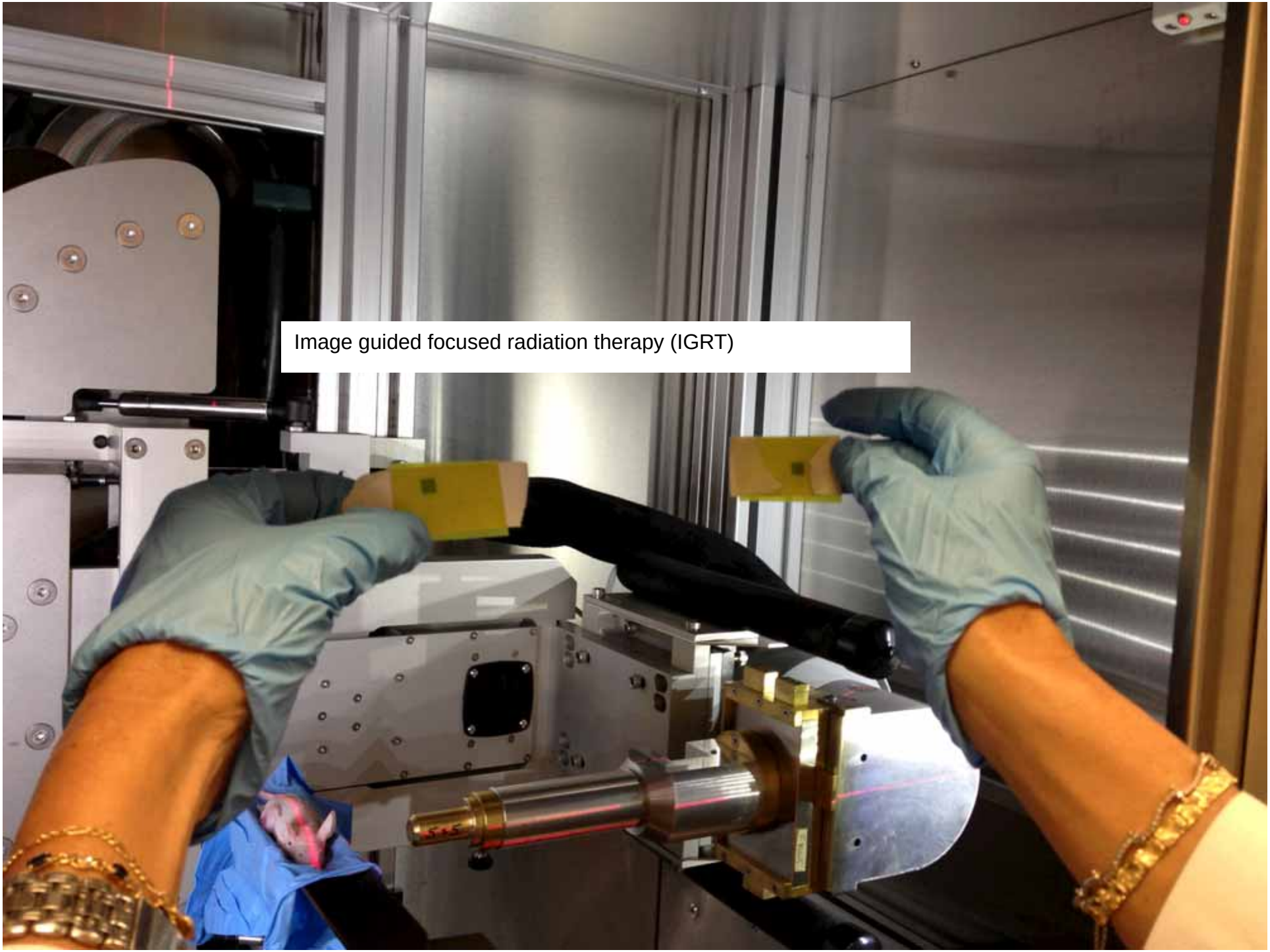
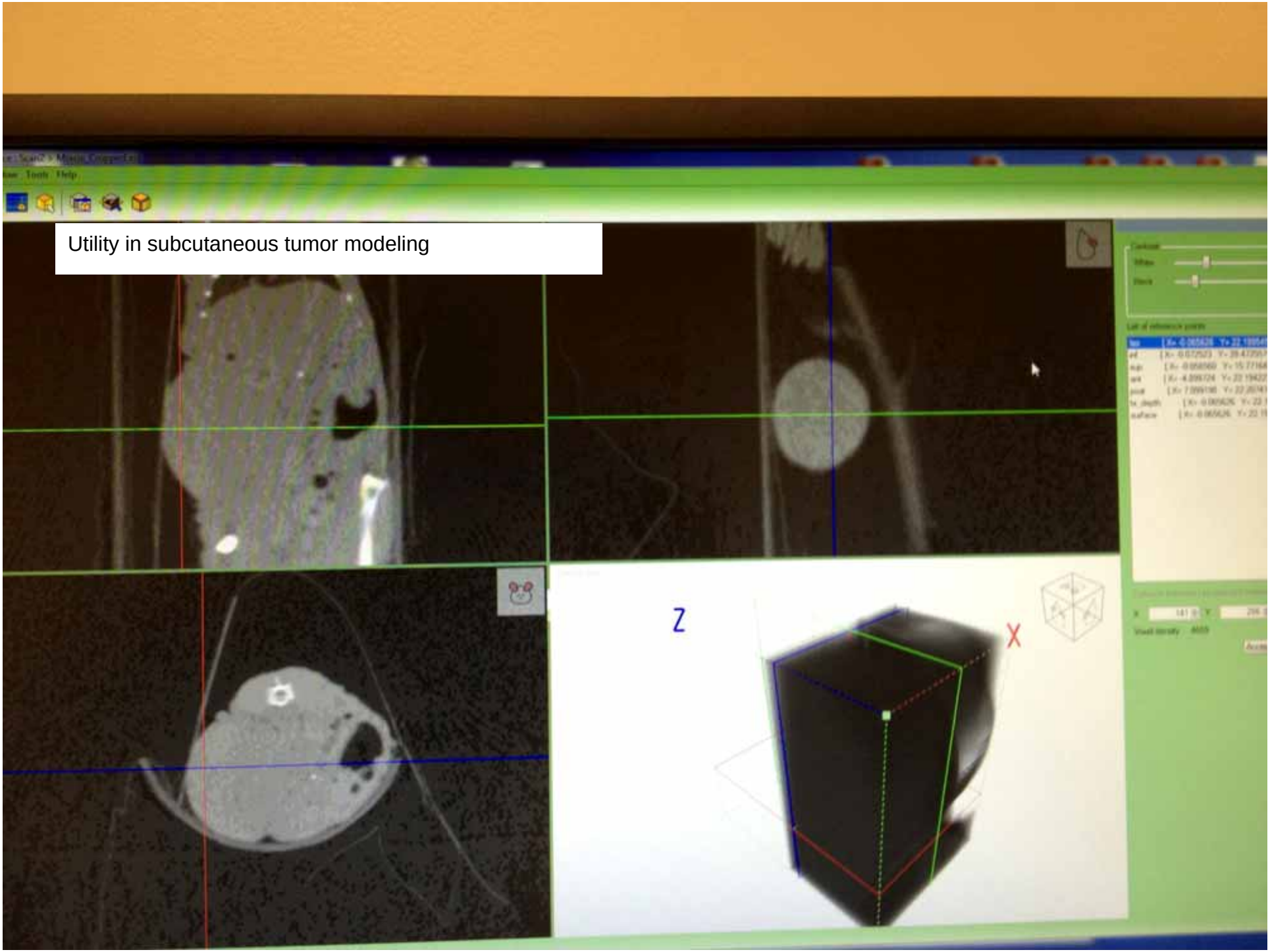
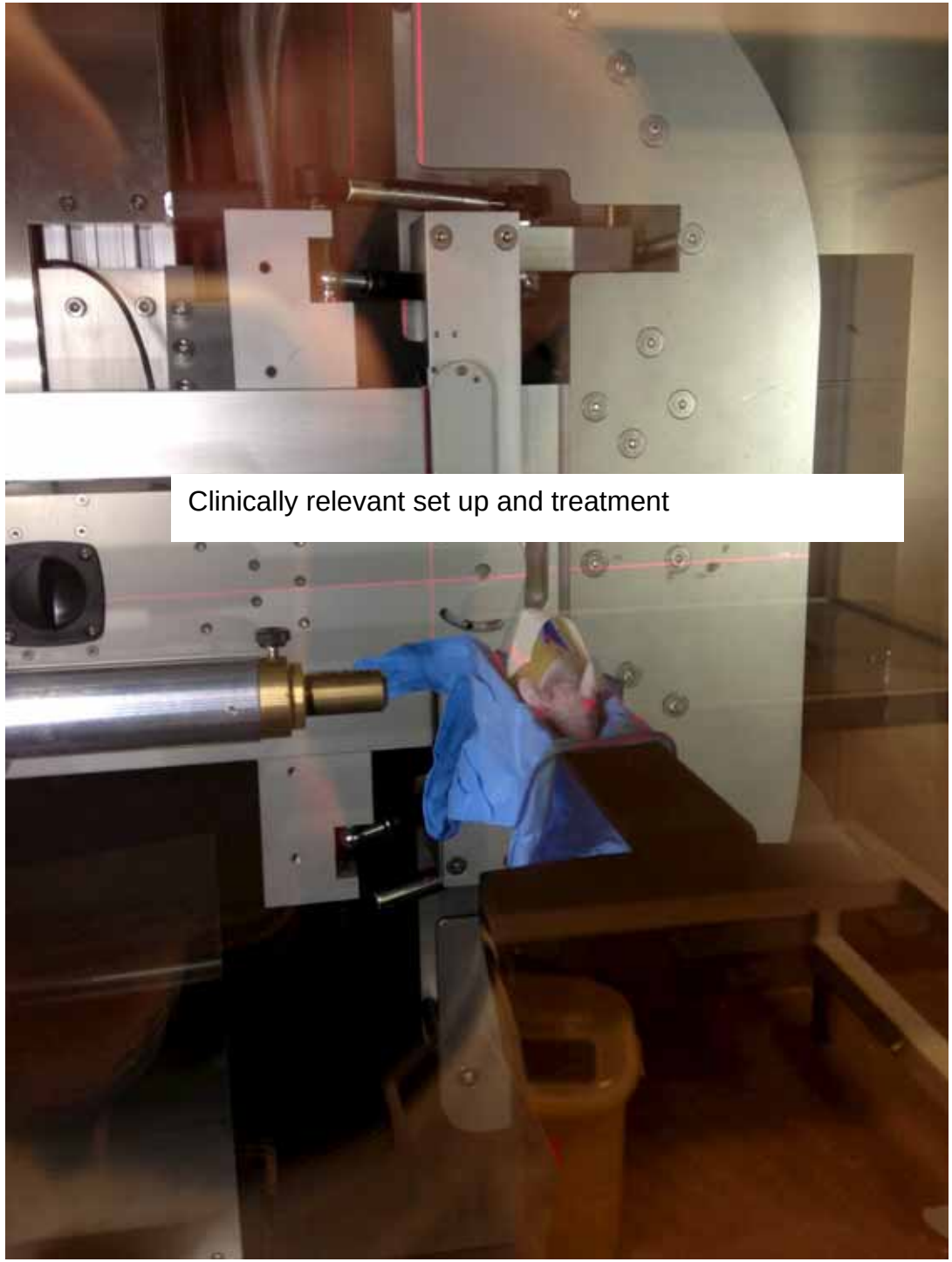


Image guided focused radiation therapy (IGRT)


Utility in subcutaneous tumor modeling
Z
X

Focused CT imaging and IGRT in subcutaneous tumor modeling



Clinically relevant set up and treatment


Video monitoring of experimental technique
McKESSON

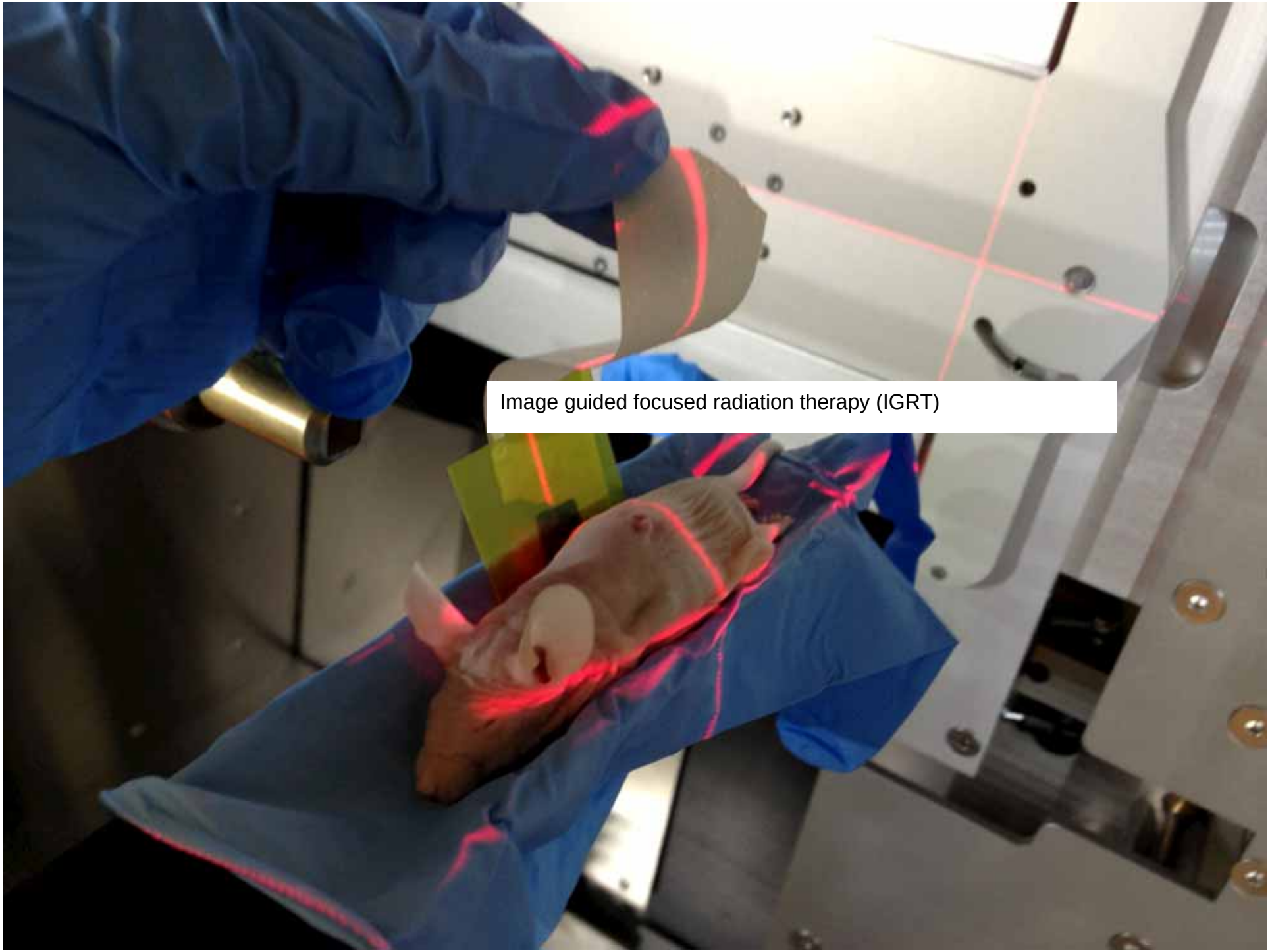
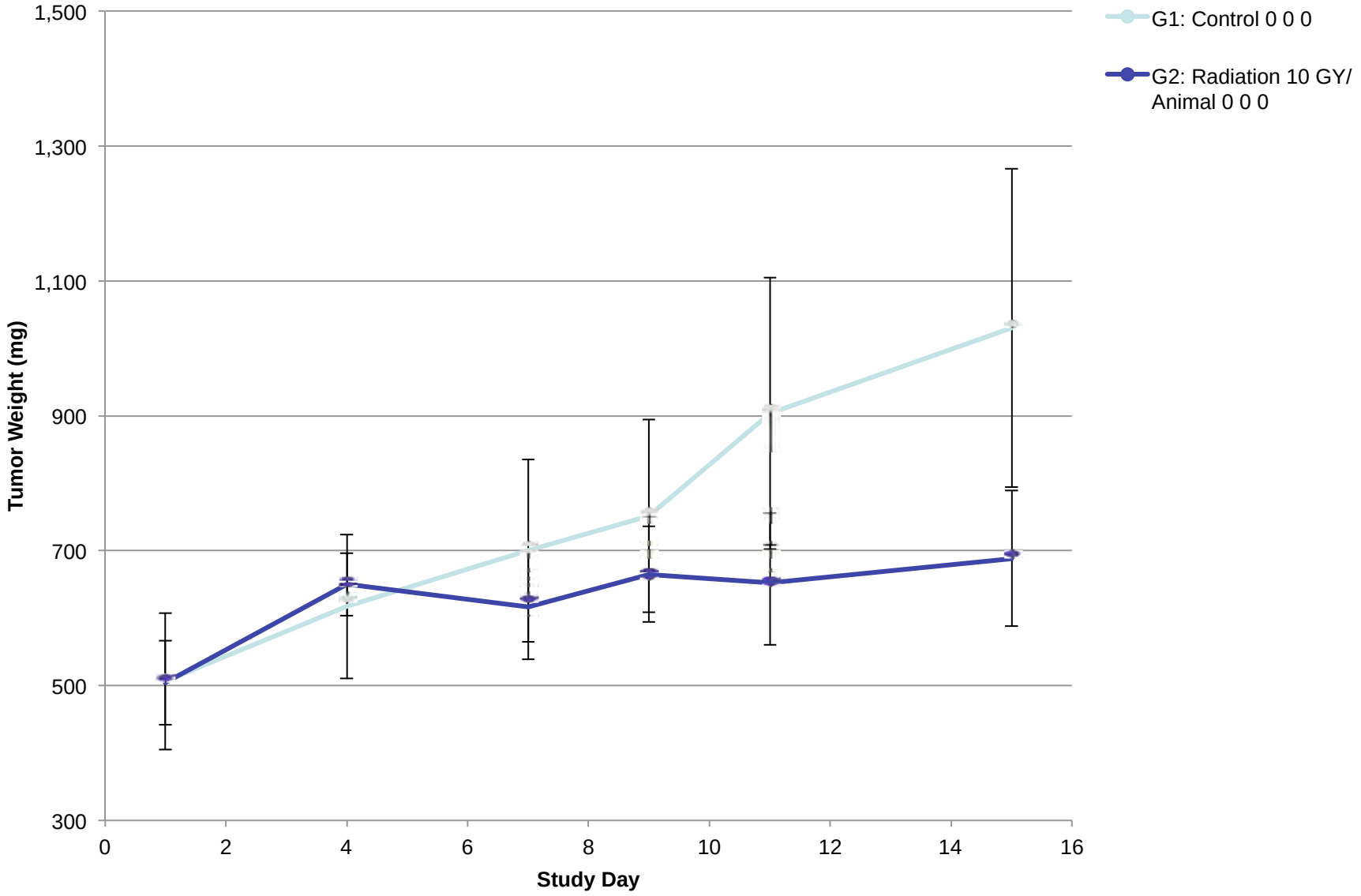


Image guided focused radiation therapy (IGRT)

Figure 1: Effect of Treatment on Tumor Weight



ARIEL's SARRP research platform is critical to identify new agents that, when used with radiation therapy, could improve outcomes for patients with cancer

Enables rapid testing of anti-cancer drugs combined with radiation therapy

Accelerates the identification of novel medicines that could increase cancer killing capabilities

Combination of TD2 leading research science and 21st Century Oncology's clinical radiation expertise can be instrumental in shaping future cancer treatment protocols and identifying active combination approaches

From Bench to the Bedside … and Back Again

Current Clinical Trials of RDI (clinical trials.gov)

Table 2: Ongoing Combination Trials

Clinical trials.gov identifier	Sponsor	Immunotherapy	Radiotherapy	Treatment timing	Phase	Condition
NCT00589875	Advantagene Inc.	AdV-tk injection into tumor bed	Standard of care	Radiotherapy 3-7 days post-injection	2	Malignant Glioma +2
NCT00634231	Advantagene Inc.	AdV-tk injection into tumor/ tumor bed	Standard of care	Radiotherapy 3-7 days post-injection	1	Malignant Glioma
NCT00751270	Advantagene Inc.	AdV-tk injection into tumor bed	Standard of care	Radiotherapy 3-7 days post-injection	1	Malignant Glioma + 2
NCT00861614	Bristol-Myers Squibb	Ipilimumab	Unspecified treatment	Radiotherapy prior to Ipilimumab	3	Castrate resistance prostate cancer
NCT01276730	James Graham Brown Cancer Center	Intergeron-a2b	40-45 Gy irradiation in conventional fractions with other dosages to follow	Interferon-a2b and retinoic acid tablets starting the first day of radiation	2	Advanced Cervical Cancer
NCT01347034	H. Lee Moffitt Cancer and Research Institute	Intratumoral injection of autologous dendritic cell	Conventional radiotherapy with boost	Radiotherapy prior to DC injection	2	Soft Tissue Sarcoma
NCT01421017	New York University School of Medicine	Topical imiquimod	6 Gy at five fractions	Evening of first radiation treatment begins imiquimod	1 / 2	Breast cancer
NCT01436968	Advantagene Inc.	AdV-tk injection + oral calacyclovir	Standard EBRT	3 injections: prior, immediately prior, and during radiotherapy	3	Prostate cancer
NCT01449279	Stanford University	Ipilimumab	Palliative Radiotherapy	Radiotherapy starts within 2 days of the first Ipilimumab dose	1	Melanoma
NCT01497808	Abramson Cancer Center (University of Pennsylvania)	Ipilimumab	Stereotactic body radiotherapy	Radiotherapy before Ipilimumab	1 / 2	Melanoma
NCT01565837	Wolfram Samlowski	Ipilimumab	Stereotactic ablative radiotherapy	Radiotherapy to 1-5 lesions after initial dose of Ipilimumab	2	Melanoma
NCT01595321	Sidney Kimmel Comprehensive Cancer Center	Cyclophos-phamide with vaccine	6.6 Gy administered over 5 days	Radiotherapy begins 7-14 days after vaccine dose and less than 12 weeks following operation	1	Pancreatic cancer

Agassi, A.M., Myslicki F.A, Shulman J.M.et al
The Promise of combining radiation therapy and immunotherapy: morbidity and toxicity. 2014 Future Medicine *in press*

Current Clinical Trials of RDI (clinical trials.gov)

Table 2: Ongoing Combination Trials (cont.).

Clinical trials.gov identifier	Sponsor	Immunotherapy	Radiotherapy	Treatment timing	Phase	Condition
NCT01689974	New York University School of Medicine	Ipilimumab	6 Gy delivered daily for 5 days	Radiotherapy begins 4 days prior to Ipilimumab	2	Melanoma
NCT01758458	Fred Hutchinson Cancer Research Center	MCPyV TAg-specific polyclonal autologous CD8-positive T cell vaccine	Unspecified treatment	Radiotherapy or injection within 1-3 days prior with repeats	1	Merkel Cell Carcinoma
NCT01807065	City of Hope Medical Center	Sipuleucel-T infusions over an hour on three different days (23rd, 36th, 50th)	EBRT in weeks one and two	Radiotherapy given one week prior to Sipuleucel-T	2	Prostate cancer
NCT01818986	University of Texas Southwestern Medical Center	Sipuleucel-T	Stereotactic Ablative Body Radiation	Unspecified	2	Metastatic Castrate-resistant Prostate Cancer
NCT01896271	University of Texas Southwestern Medical Center	High dose IL-2	Stereotactic Ablative Body Radiation	Unspecified	2	Metastatic Renal Cancer
NCT02086721	Maastricht Radiation Oncology	L 19-IL2	Stereotactic Ablative Body Radiation	Scheduled dose of SABR and cycles of L19-IL2	1	Oligometastatic non-small cell lung cancer patients
NCT02107430	Sotio a.s.	DCVAC/PCa	Unspecified treatment	DCVAC/PCa post radiotherapy	2	Prostate cancer

Agassi, A.M. et al.

TRC TRANSLATIONAL RESEARCH CONSORTIUM

21C 2013-2: Provenge and Radiation Therapy

Principal Investigator: Steven Eric Finkelstein, MD

Co-Principal Investigator: Constantine Mantz, MD

Co-Principal Investigator: Neal Shore, MD

Institution(s): 21st Century Oncology Translational Research Consortium (TRC)

21st Century Oncology

TRC TRANSLATIONAL RESEARCH CONSORTIUM

Introduction

- Background
  - Prostate Cancer
    - 240,890 new cases and 33,720 related deaths in 2011
    - Approximately 80% of prostate cancer cases are diagnosed when the cancer is still confined to the primary site
    - 20% to 40% of these subjects will eventually experience disease recurrence

Introduction

- In men with recurrent disease, androgen deprivation therapy (ADT) is the current standard of care for androgen-dependent advanced prostate cancer and achieves temporary tumor control or regression in approximately 80% of subjects
- Until recently, docetaxel was the only therapy to have demonstrated an improvement in overall survival in mCRPC patients

Introduction

- Research Hypothesis:
  - Radiation in combination with Provenge based immunotherapy may improve outcomes seen on imaging as well as immunologic monitoring (as per PRIME).

Sipuleucel-T

- Sipuleucel-T (Provenge®), an autologous cellular immunotherapy product designed to stimulate an immune response against prostate cancer

Sipuleucel-T (Provenge®)

Current Status of Sipuleucel-T for the Treatment of Asymptomatic or Minimally Symptomatic Metastatic Castration-Resistant (Hormone- Refractory) Prostate Cancer

Steven E Finkelstein, MD1 and Mayer Fishman, MD, PhD2

- 121st Century Oncology Translational Research Consortium, Scottsdale, AZ; and 2Department of Genitourinary Oncology, Moffitt Cancer Center, Tampa, FL, USA
- *Int J Clin Rev* 2011;12:10 doi: 10.5275/ijcr.2011.12.10
- **In April 2010, the US Food and Drug Administration approved sipuleucel-T, an autologous cell infusion for anticancer treatment of minimally symptomatic prostate cancer based on the stimulation of an immune attack using AP8015, a prostatic acid phosphatase (PAP)/granulocyte–macrophage colony stimulating factor antigen. The results of randomized trials of sipuleucel-T, with data collected over a decade, showed consistent improvements in the median overall survival of patients who received the treatment compared with control patients. Control patients were administered autologous leukocytes that were not processed with the proprietary *ex vivo* stimulation treatment. Time to disease progression and change in prostate-specific antigen level were not significantly different between patients receiving sipuleucel-T and control patients. In immunological studies, increased PAP-specific T cell titers and antibody titers were demonstrated following the treatment. In this review, the current, developing status of sipuleucel-T in prostate cancer therapy is described.**

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Efficacy of Sipuleucel-T

- Randomized, double-blinded, phase 3 trial (D9901, N=127)
  - men with asymptomatic mCRPC
    - 41% reduction in the risk of death relative to those randomized to a control product manufactured from autologous PBMCs without activation with PA2024
    - median survival was 25.9 months for subjects randomized to sipuleucel-T compared with 21.4 months for those randomized to control
    - 31% reduction in the risk of disease progression

Efficacy of Sipuleucel-T

- Second study with the same design as D9901 (N=98)
  - –21% reduction in the risk of death for subjects randomized to sipuleucel-T

Efficacy of Sipuleucel-T

- IMPACT trial (D9902B, N=512 randomized 2:1 to receive Sipuleucel-T vs. Control)
  - –22.5% reduction in the risk of death relative to those subjects randomized to the control arm
  - –The median survival in the sipuleucel-T arm was 25.8 months vs. 21.7 months in the control arm

Safety of Sipuleucel-T

- Most common adverse events (AE's) have been temporally related to APC product infusion
  - The most common AEs observed in ≥ 5% of sipuleucel-T subjects, and at a rate at least twice that of control subjects, included chills, pyrexia, headache, myalgia, influenza-like illness, and hyperhidrosis
    - The majority of these events occurred within 1 day of infusion, were Grade 1 or 2 in severity, and were generally of short duration
  - Grade 3 or Grade 4 events were reported in 27.6% of subjects in the sipuleucel-T group, compared with 28.4% in the control group.
  - Cerebrovascular events (CVEs) occurred in 3.5% of subjects in the sipuleucel-T group compared with 2.6% in the control group
    - CVE risk with sipuleucel-T treatment is being further evaluated in Study P10-3 (PROCEED), a registry of approximately 1,500 patients

Radiation Therapy

- induction of DNA damage in the neoplastic cells
  - accumulation of DNA breaks and consequent insufficient repair is the trigger for pathways including Bcl2 family apoptotic and anti-apoptotic proteins, p53 dependent, and independent pathways, or TRAIL (tumor necrosis factor [TNF] TNF-Related Apoptosis-Inducing Ligand) dependent mechanisms

Radiation Therapy

- Besides the phenomenon of cells dying within an irradiated tumor, several processes have specific relevance to immunotherapy
  - The most dramatic clinical outcome is the abscopal effect

Radiation Therapy

- The Abscopal Effect
  - Regression of a distant tumor mass
    - Less apparent outcomes, still with major clinical impact, may occur as well
      - accelerating or completing definitive clearance of the tumor which was being irradiated
      - clearance of other metastatic disease that was not clinically apparent because it was microscopic

Radiation Therapy

- Radiation Effects – The Lymphocytes
  - There is not significant systemic lymphopenia from prostate cancer external beam radiation therapy, our group has observed
  - Others suggest that hypofractionated radiation therapy can mediate in CD4+ and CD8+ lymphocyte number, but not of natural killer (NK) and of B lymphocytes
  - This effect was counterbalanced in those patients receiving combined androgen blockade, with goserlin and flutamide, suggesting a converse effect of testosterone suppression

Radiation Therapy

- Radiation Effects – Dendritic Cells
  - The tumor microenvironment has potential to modulate the phenotype of dendritic cells (DC) to favors the pathologic tolerance for the tumor
  - Irradiated DC would still stimulate T cell proliferation in the MLR (mixed lymphocyte reaction) assay but at a lower level, and with higher T cell production of IL-2 and IL-4

Imaging for Prostate Cancer

Radiologic Techniques for Identification of Prostate Cancer Metastases

Techniques	Pros	Sensitivity and Specificity
CT	Staging Small LN may be missed	Sensitivity broad variation Specificity broad variation
MRI	Zonal anatomy Extracapsular extension Seminal vesicle invasion	Sensitivity 75% Specificity high
99mTc-MDP Scintigraphy	Advanced active osteoblastic activity	Sensitivity 40-94% Specificity 89% Negative results do not rule out bone mets
18F-Sodium Fluoride PET/CT	More subtle osteoblastic activity	Sensitivity 95% Specificity 95% Difficulty post treatment as lesions may remain active for long period due to ongoing bone remodeling
C11-Choline PET/CT	Imaging lipid membrane synthesis via up-regulation of choline kinase. Identifies local recurrences, small nodes and bone lesions	Dependent on PSA level. 74% overall detection rate, 86% above 2.0ng/mL. Limited value with PSA <2.0ng/mL. Single site use FDA approval [1]
C11-Acetate PET/CT	Imaging lipid membrane synthesis via up-regulation of fatty acid synthase. Identifies local recurrences, small nodes and bone lesions not identified on other imaging	Dependent on PSA level. 84% overall detection rate, 90% above 2.0ng/mL. Performs better than choline at PSA < 2.0ng/mL (77%) [2,3]

1. Mitchell, C. R., V. J. Lowe, et al. (2013). "Operational characteristics of (11)c-choline positron emission tomography/computerized tomography for prostate cancer with biochemical recurrence after initial treatment." *J Urol* 189(4): 1308-1313.
2. Almeida, F. (2011). PET Imaging Characteristics of C11-Acetate in Patients With Recurrent Prostate Carcinoma. *Arizona Molecular Imaging Center*, NCT01304485
3. Almeida, F., Yen, CK., Finkelstein, F. “Early imaging improves performance of C11-Acetate PET/CT for recurrent prostate adenocarcinoma”. *UroToday International Journal* – in Press

Imaging for Prostate Cancer

- F18 PET/CT Bone Scan and C11 Acetate PET/CT
  - Advanced imaging in prostate cancer, especially in slow-growing or well differentiated tumors which are not FDG avid

F18 PET/CT Imaging for Prostate Cancer

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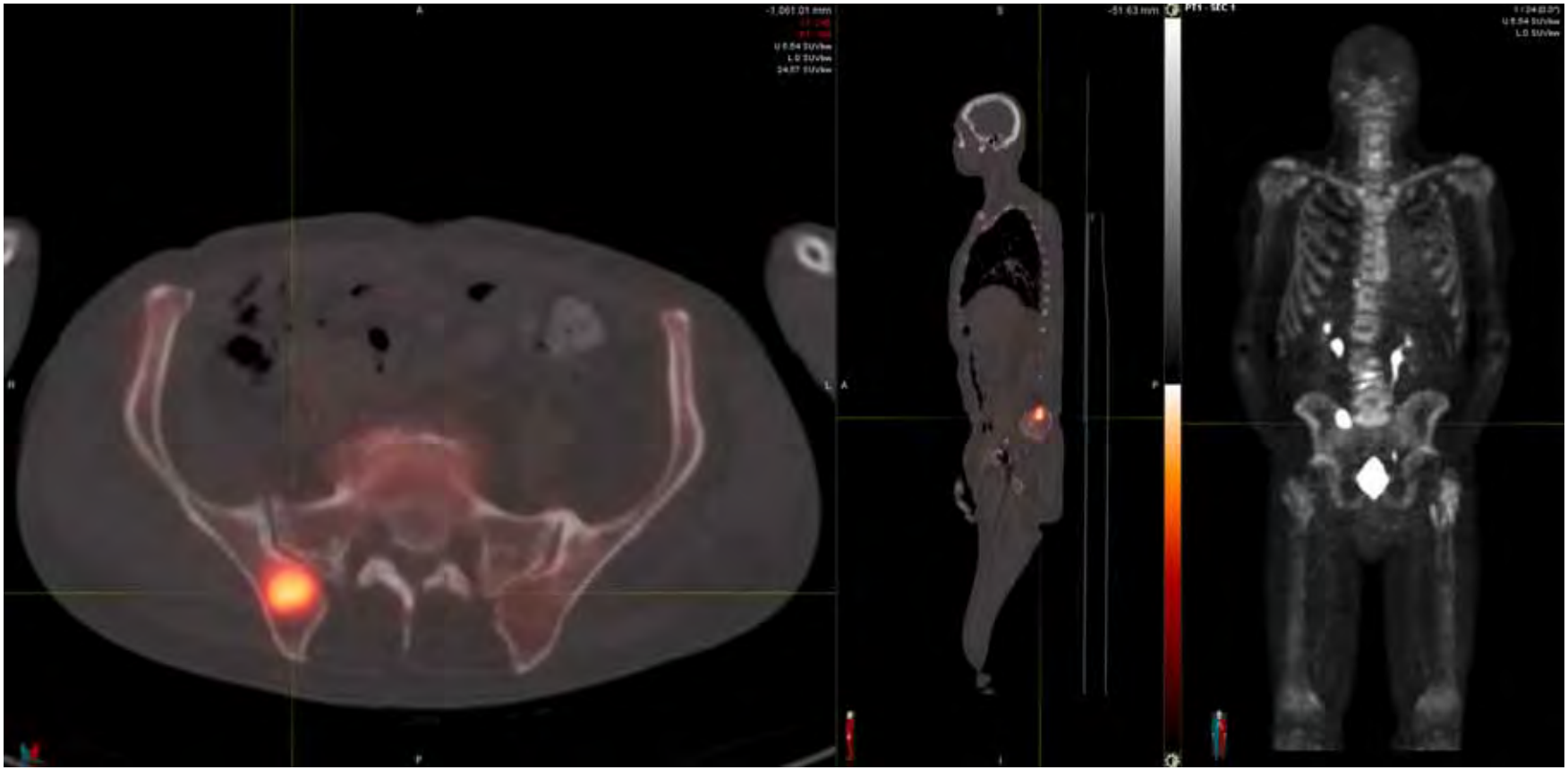
F18 PET/CT Imaging for Prostate Cancer



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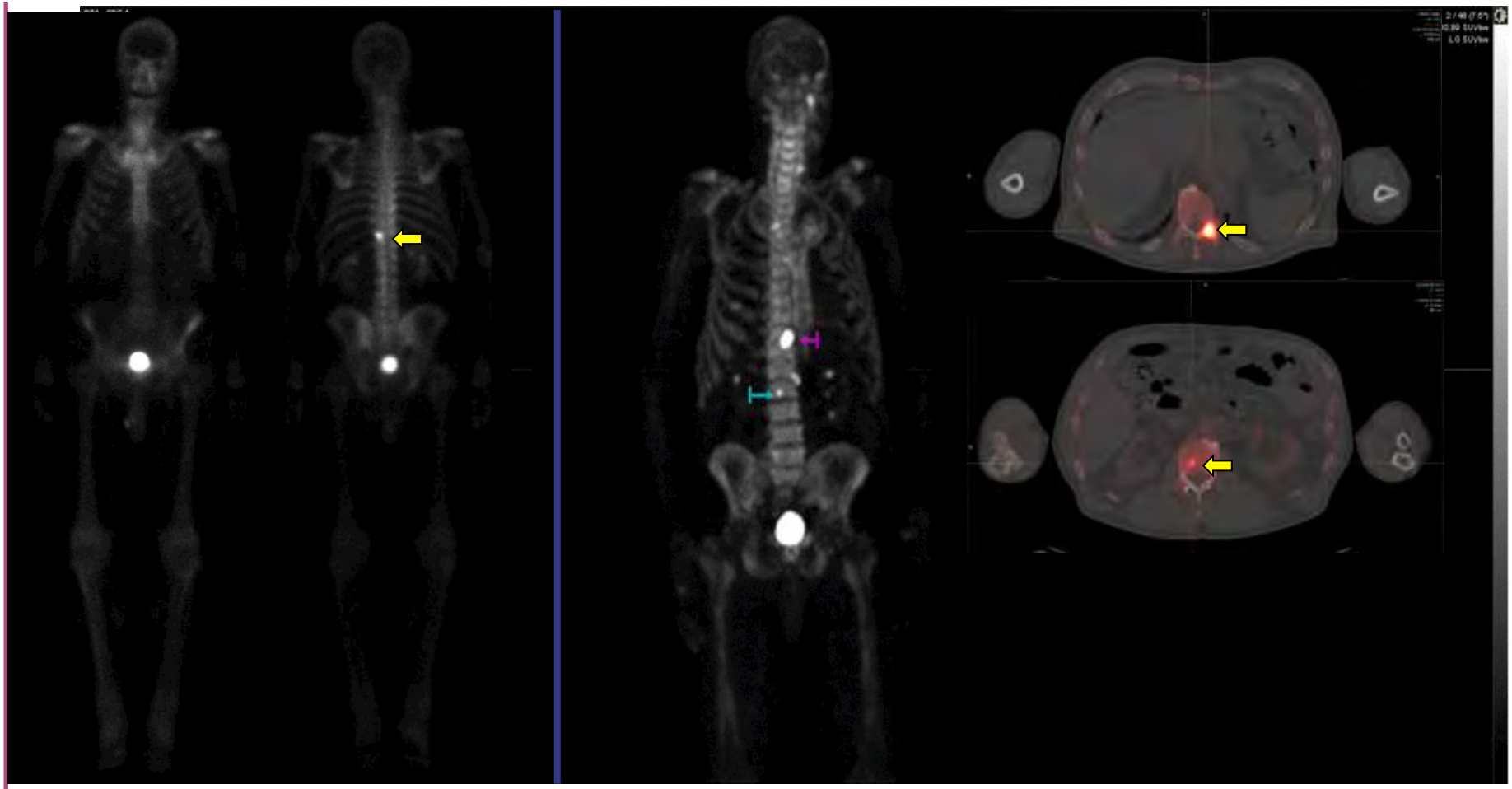
F18 PET/CT Imaging for Prostate Cancer



PSA 0.84ng/mL

Left Images: Anterior & Posterior Tc99MDP Bone scan – interpreted as equivocal and probably DJD in posterior element of spine.

Right Images: NaF18 PET/CT bone scan (3D imaging with direct fusion to CT) – lesion in lower thoracic spine can be seen to involve the left pedicle and lamina, indicative that this is a metastasis and not DJD. Additional 3mm bone metastasis is seen in L2 which can not be seen on Tc99 scan

C11 Acetate PET/CT Imaging for Prostate Cancer

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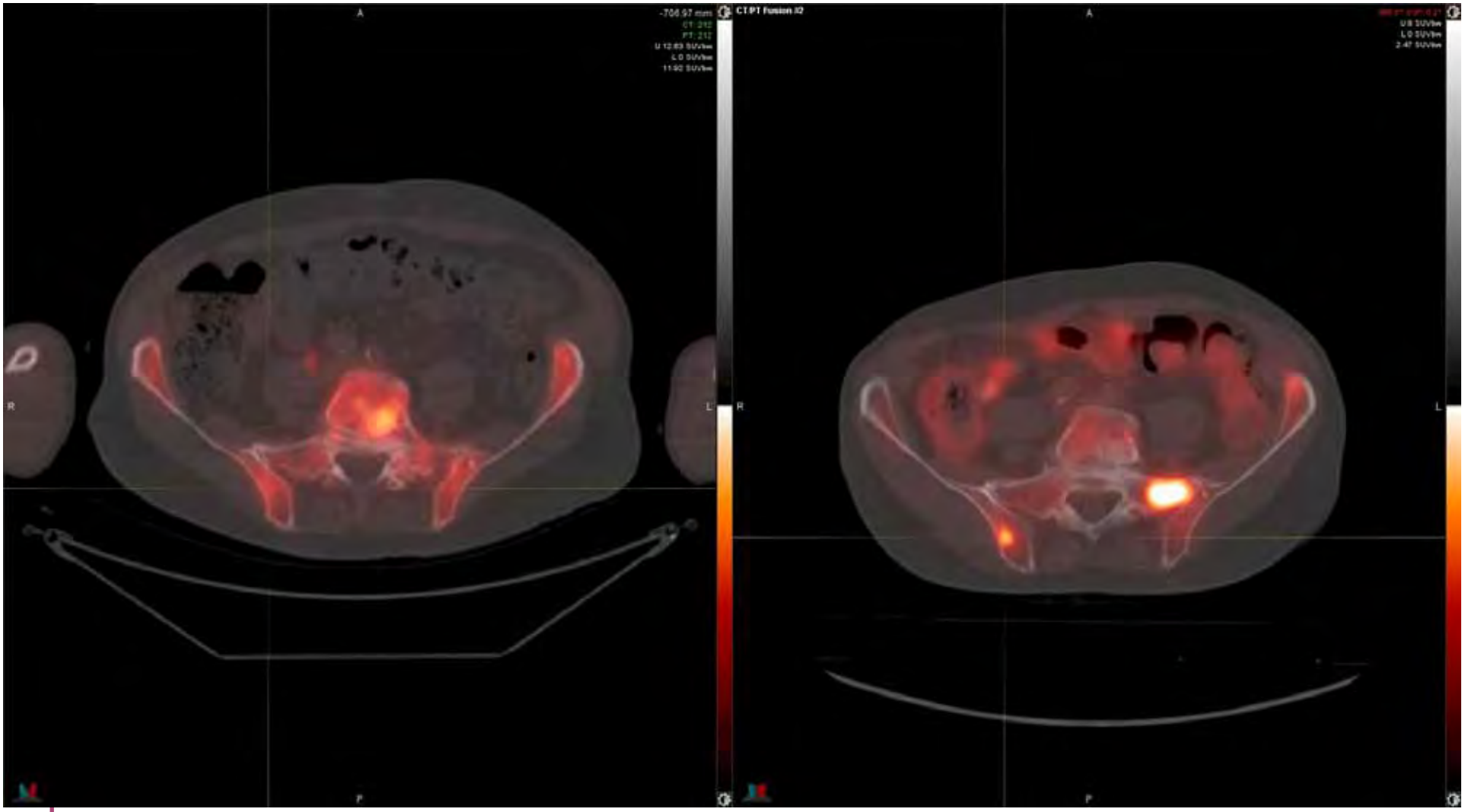
Imaging for Prostate Cancer

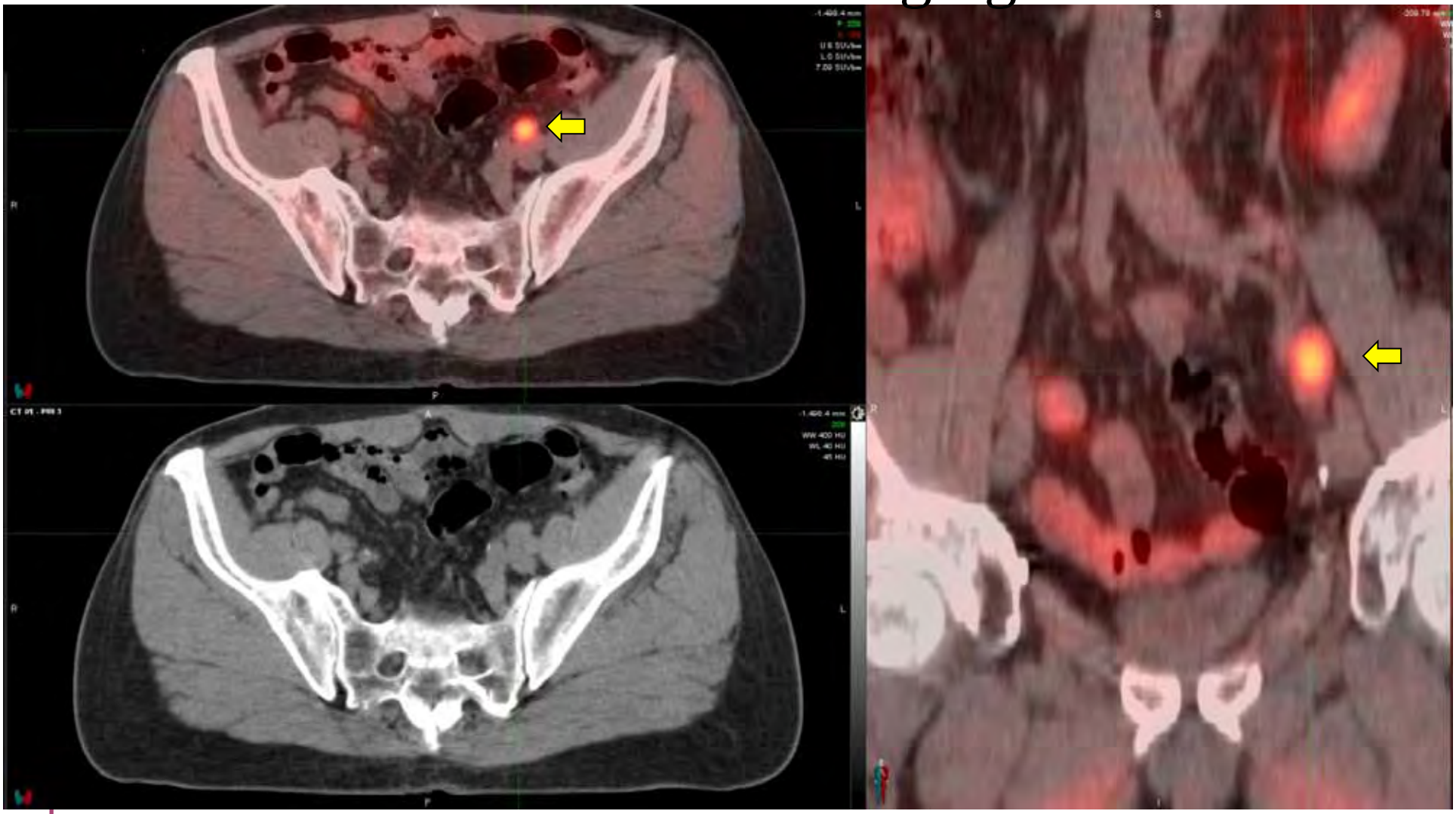
- C11-Acetate was been used for cardiac studies to measure myocardial oxygen consumption with no reported adverse effects
- Several published studies have been performed with C11-Acetate in the evaluation of prostate cancer and prostate cancer recurrence in humans

C11 Acetate PET/CT Imaging for Bone



RP 10 years previously (Gs 8, PSA 6.4). Rising PSA = 43.5 ng/mL.
F18Na bone scan left compared to C11-Acetate right performed 1 day apart.
C11 shows intramedullary pelvic lesions not well seen on F18 scan

C11 Acetate PET/CT Imaging for LN



Gs 7 (4+3). PSA 4.6. RP w/ ECE & B/L SV involvement. Post RP @ 9 months was PSA 0.42 ng/mL. Single 1.5 cm metabolic left external iliac node. Pt underwent ADT + Salvage RT (Bed & Pelvic Nodes). C11 altered plan beyond standard RTOG PF-CTV (prostate fossa clinical target volume). PSA now <0.1ng/mL

C11-Acetate PET/CT

Author	Year	n	PSA ng/mL	Detection rate
Kotzerke [17]	2002	31	0.1 – 150.6 (mean 10.4)	83%
			<2.0	63%
Frickle [18]	2003	25	0.3 – 400 (mean 50)	83%
Oyama [15]	2003	46	0.3 – 47.5 (mean 5.2)	59%
Sandblom [22]	2006	20	Median 2.0	75%
Vees [23]	2006	11	<0.8	55%
Wachter [24]	2006	50	0.5- 24.9	64%
Albrecht [25]	2007	17 (RT)	2.6-30.2	82%
		15 (RP)	0.08-4.8	60%
Dusing [26]	2010	20	?	85%
Yu [27]	2011	8	6.3 – 2,012	100%
Haseebuddin [28]	2013	107	1.4-225.4	68%
Almeida et al.	2014	200		Stay tuned …

Prior studies have demonstrated a wide range of detection rates, but when reviewed in context of technique and PSA, generally good detection rates are found.

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21C 2013-2 Study Objectives

- This study will assess the effect of standard of care radiation therapy to augment anti-tumor responses from the standard of care immune therapy, Sipuleucel-T (Provenge®).

Study Design

- This is a multicenter trial enrolling men with advanced prostate cancer who are to receive combination radiation and Sipuleucel-T (Provenge®)
- 100 patients to be enrolled
- Study participation will end following 36 months of follow-up

Study Design

- Immune monitoring and F18 PET CT (in all patients) and C11 Acetate PET CT (in 30/100 patients where technology available) baseline within 90 days starting radiation therapy
- Patients will undergo radiation to area of concern per standard of care followed by Sipuleucel-T (Provenge®)

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TRC TRANSLATIONAL RESEARCH CONSORTIUM

Study Design

- Immune monitoring and imaging to include F18 PET CT and C11 acetate PET CT will be completed post radiation prior to Sipuleucel-T (Provenge®) and at 6 and 26 weeks post last infusion of Sipuleucel-T (Provenge®)
- Additional follow-up visits will be completed at 39 and 52 weeks and 15, 18, 21, 24, 27, 30, 33 and 36 months

Subject Selection Criteria

- Inclusion Criteria
  - Be at least 18-years-old or older
  - Signed and dated IRB-approved Informed Consent form for the study.
  - Received an explanation of the study, including satisfactory answers to all questions related to the proposed research.
  - Is undergoing physician directed radiation treatment.
  - Eligible for Sipuleucel-T (Provenge®)

Subject Selection Criteria

- Exclusion Criteria
  - Patient on systemic immunosupressive agent
  - The potential subject is unwilling or hesitant to participate for any reason and/or fails to complete the appropriate Informed Consent Form.

Study Calendar

Provenge and Radiation Therapy								
Study Calendar								
Site:								
Assessments	**Screening (within 90 days of RT Start)**	**Radiation Treatment**	**6 weeks post last infusion**	**26 weeks post last infusion**	**39 week Follow Up**	**52 Week Follow Up**	**15, 18, 21, 24, 27, 30, 33 mo Follow Up**	**36 mo Follow Up/End of study**
Informed Consent	X							
Inclusion/Exclusion Criteria	X							
Physical Exam (including KPS or ECOG)	X			X	X	X	X	X
Medical History	X			X	X	X	X	X
F18 PET CT	X		X	X				
C11 Acetate PET CT	X		X	X				

Endpoints

- Endpoints
  - The primary endpoint to compare immune stimulation in patients receiving radiation to lesions followed 28 days later by Sipuleucel-T (Provenge®) to patients who receive Sipuleucel-T (Provenge®) alone in Phase IV PRIME study
- Secondary endpoints
  - To determine if qualitative and quantitative changes in imaging uptake occur in response to radiation and Sipuleucel-T (Provenge®)
  - To quantify survival in all subjects as compared with PROCEED and PRIME
- Exploratory objective
  - To compare the sensitivity and specificity of C11 acetate PET/CT and F18 PET/CT imaging modalities in patients treated with radiation and sipuleucel-T

Statistical Methodology

- Primary Endpoint
  - The percentage of subjects who exhibit a two-fold increase in peripheral immune response at any post-treatment time point (6, 10, 14, 26, 39 and 52 weeks after the first infusion of sipuleucel-T) utilizing IFNγ ELISPOT response to PA2024.
- Secondary endpoint
  - To determine if qualitative and quantitative changes in imaging uptake occur in response to systemic Provenge therapy
- Clinical Response Determination
  - Clinical symptoms, bone scans, CT scans, and PSA levels will be reviewed, and the patients designated as demonstrating a response to therapy, stable disease, or progressive disease. Sensitivity, specificity, PPV, NPV and accuracy of F18 and C11 Acetate PET will be determined.

Current status

- 21C 2013-2 Open and accruing in Arizona and Florida
- Clinical trials.gov
- Accrual 15/100

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